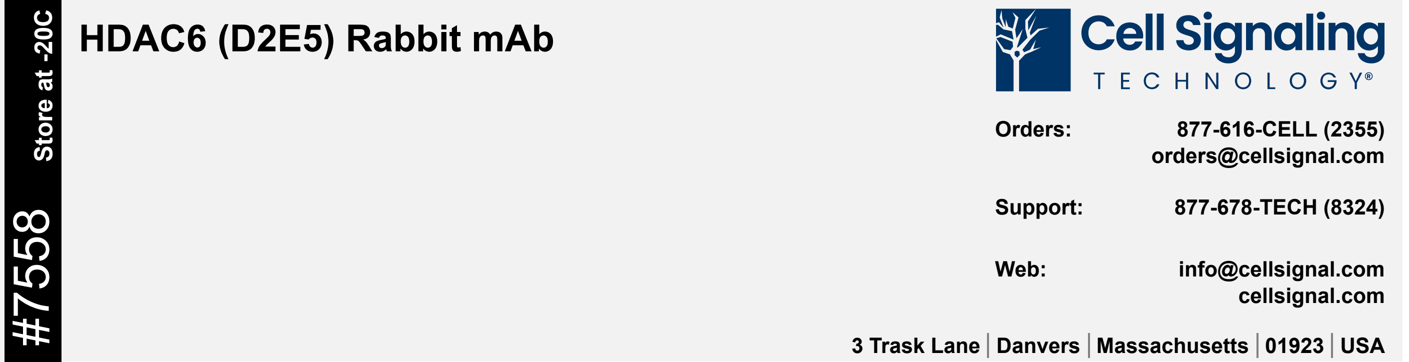
Revision 3



HDAC6 (D2E5) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IHC-P, IF-IC, FC-FP	H Mk	Endogenous	160	Rabbit IgG	#Q9UBN7	10013

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:200 - 1:800
Immunofluorescence (Immunocytochemistry)	1:100 - 1:400
Flow Cytometry (Fixed/Permeabilized)	1:100 - 1:400

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

HDAC6 (D2E5) Rabbit mAb recognizes endogenous levels of total HDAC6 protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a recombinant protein specific to the carboxy terminus of human HDAC6 protein.

Background

HDAC6 is a class II histone deacetylase enzyme localized to the cytoplasm and associated with the microtubule network (1). It is involved in the regulation of many cellular processes, including cell migration, immune synapse formation, viral infection, and degradation of misfolded proteins (1). HDAC6 contains two tandem catalytic domains that facilitate the deacetylation of multiple protein substrates, including histones and non-histone proteins such as tubulin, cortactin, and HSP90. Despite the ability to deacetylate histone proteins *in vitro*, there is no evidence for HDAC6-mediated deacetylation of histones *in vivo* (2,3). The acetylation/deacetylation of tubulin on Lys40 regulates binding and motility of the kinesin-1 motor protein and subsequent transport of cargo proteins such as JNK-interacting protein 1 (JIP1) (4). The acetylation/deacetylation of cortactin regulates cell motility by modulating the binding of cortactin to F-actin (5). Acetylation/deacetylation of HSP90 modulates chaperone complex activity by regulating the binding of an essential cochaperone protein, p23 (6,7). In addition to its role as a protein deacetylase, HDAC6 functions as a component of the aggresome, a proteinaceous inclusion body that forms in response to an accumulation of misfolded or partially denatured proteins (8). Formation of the aggresome is a protective response that sequesters cytotoxic protein aggregates for eventual autophagic clearance from the cell. HDAC6 contains a zinc finger ubiquitin-binding domain that binds both mono- and poly-ubiquitinated proteins (8). HDAC6 binds to both poly-ubiquitinated misfolded proteins and dynein motors, facilitating the transport of misfolded proteins to the aggresome (9,10). HDAC6 is also required for subsequent recruitment of the autophagic machinery and clearance of aggresomes from the cell (11). Thus, HDAC6 plays a key role in the protection against the deleterious effects of pathological protein aggregation that occurs in various diseases, such as neurodegenerative Huntington's disease (11).

Background References

1. Boyault, C. et al. (2007) *Oncogene* 26, 5468-76.
2. Haggarty, S.J. et al. (2003) *Proc Natl Acad Sci U S A* 100, 4389-94.
3. Zhang, Y. et al. (2003) *EMBO J* 22, 1168-79.
4. Reed, N.A. et al. (2006) *Curr Biol* 16, 2166-72.
5. Zhang, X. et al. (2007) *Mol Cell* 27, 197-213.
6. Kovacs, J.J. et al. (2005) *Mol Cell* 18, 601-7.
7. Murphy, P.J. et al. (2005) *J Biol Chem* 280, 33792-9.
8. Seigneurin-Berny, D. et al. (2001) *Mol Cell Biol* 21, 8035-44.
9. Kawaguchi, Y. et al. (2003) *Cell* 115, 727-38.
10. Boyault, C. et al. (2006) *EMBO J* 25, 3357-66.
11. Iwata, A. et al. (2005) *J Biol Chem* 280, 40282-92.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin)
IF-IC: Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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Revision 3

#7558

HDAC6 (D2E5) Rabbit mAb

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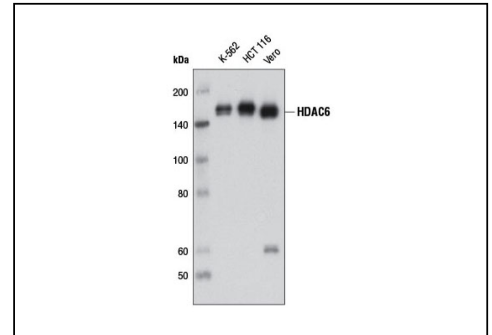
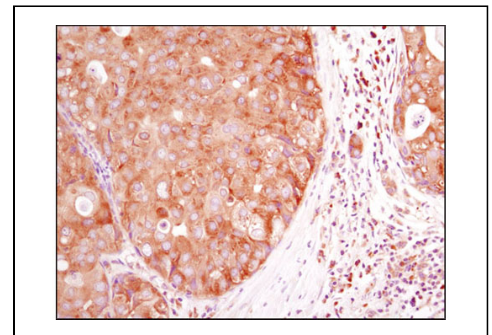
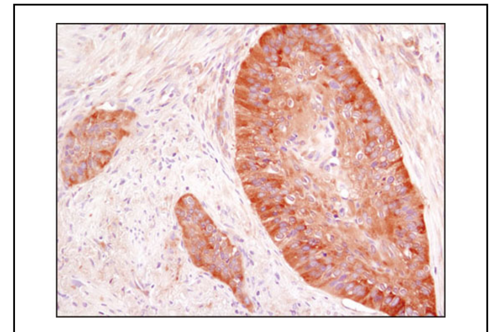


Western blot analysis of extracts from various cell lines using HDAC6 (D2E5) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using HDAC6 (D2E5) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using HDAC6 (D2E5) Rabbit mAb.

Revision 3

#7558

HDAC6 (D2E5) Rabbit mAb

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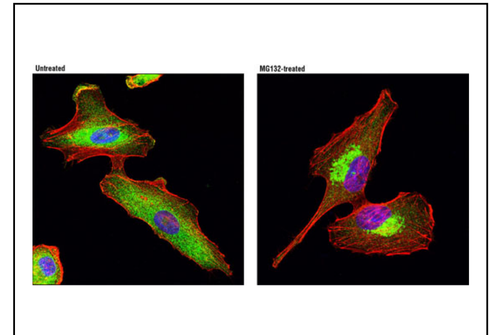
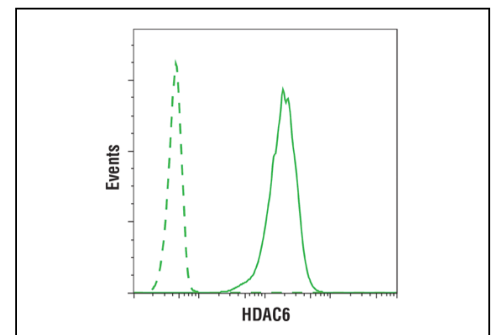
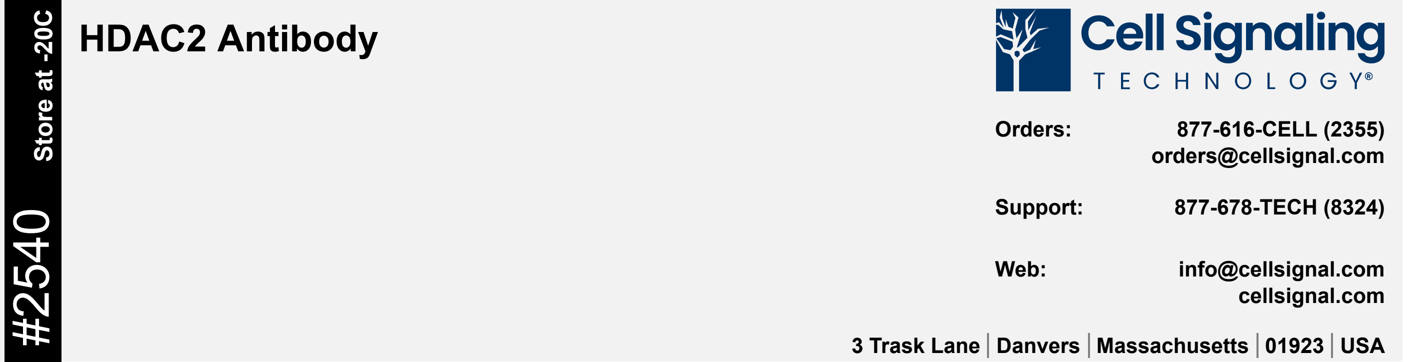


Confocal immunofluorescent analysis of A549 cells, untreated (left) or treated with MG132 (5 µM, 24 hr; right), using HDAC6 (D2E5) Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5®#4084 (fluorescent DNA dye).



Flow cytometric analysis of K562 cells using HDAC6 (D2E5) Rabbit mAb (solid line) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed line). Anti-rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

Revision 1



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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, IF-IC	H M R Mk	Endogenous	60	Rabbit	#Q92769	3066

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

HDAC2 Antibody detects endogenous levels of HDAC2 protein. The antibody does not cross-react with other HDAC proteins.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of the human HDAC2 protein. Antibodies are purified by peptide affinity chromatography.

Background

Acetylation of the histone tail causes chromatin to adopt an "open" conformation, allowing increased accessibility of transcription factors to DNA. The identification of histone acetyltransferases (HATs) and their large multiprotein complexes has yielded important insights into how these enzymes regulate transcription (1,2). HAT complexes interact with sequence-specific activator proteins to target specific genes. In addition to histones, HATs can acetylate nonhistone proteins, suggesting multiple roles for these enzymes (3). In contrast, histone deacetylation promotes a "closed" chromatin conformation and typically leads to repression of gene activity (4). Mammalian histone deacetylases can be divided into three classes on the basis of their similarity to various yeast deacetylases (5). Class I proteins (HDACs 1, 2, 3, and 8) are related to the yeast Rpd3-like proteins, those in class II (HDACs 4, 5, 6, 7, 9, and 10) are related to yeast Hda1-like proteins, and class III proteins are related to the yeast protein Sir2. Inhibitors of HDAC activity are now being explored as potential therapeutic cancer agents (6,7). HDAC1 and HDAC2 are highly homologous and are involved in histone deacetylation, chromatin remodeling and transcriptional repression (8-10). Both proteins are found together in numerous complexes including the nucleosome remodeling and deacetylation complex (NuRD), MeCP1, and the mSin3A corepressor complex.

Background References

1. Marmorstein, R. (2001) *Cell Mol Life Sci* 58, 693-703.
2. Gregory, P.D. et al. (2001) *Exp Cell Res* 265, 195-202.
3. Liu, Y. et al. (2000) *Mol Cell Biol* 20, 5540-53.
4. Cress, W.D. and Seto, E. (2000) *J Cell Physiol* 184, 1-16.
5. Gray, S.G. and Ekström, T.J. (2001) *Exp Cell Res* 262, 75-83.
6. Thiagalingam, S. et al. (2003) *Ann. N.Y. Acad. Sci.* 983, 84-100.
7. Vigushin, D.M. and Coombes, R.C. (2004) *Curr Cancer Drug Targets* 4, 205-18.
8. Zhang, Y. et al. (1999) *Genes Dev.* 13, 1924-1935.
9. Ng, H.H. et al. (1999) *Nat. Genet.* 23, 58-61.
10. Zhang, Y. et al. (1997) *Cell* 89, 357-364.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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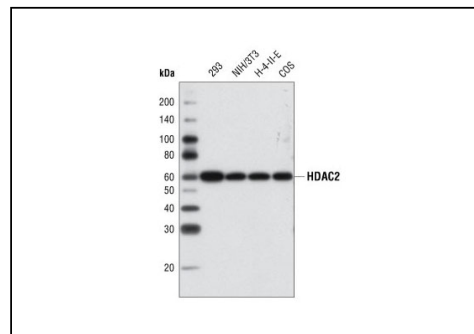
Revision 1

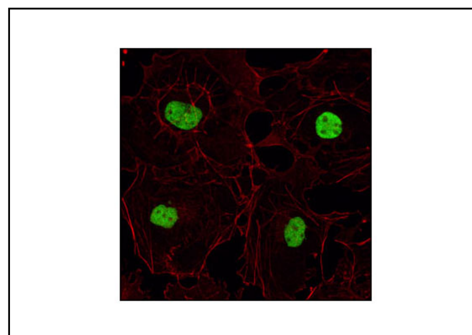
#2540

HDAC2 Antibody

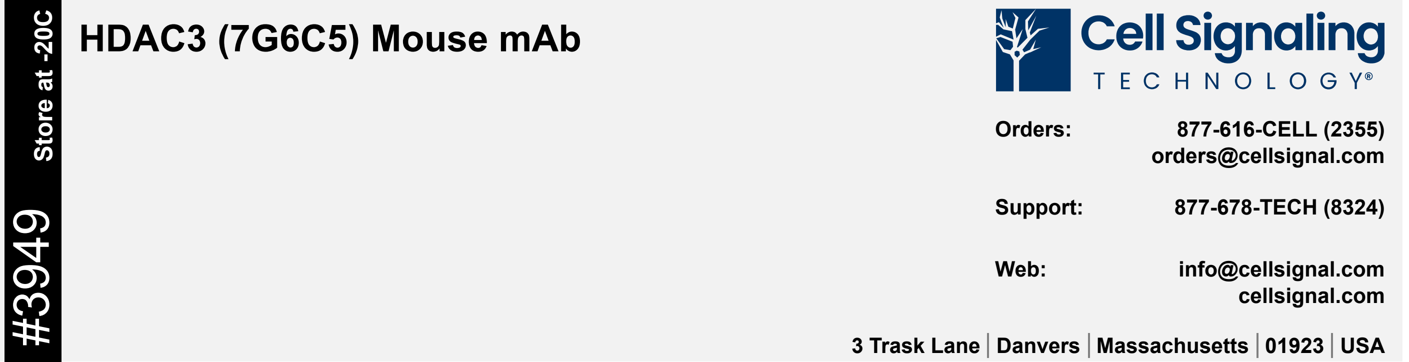
Western blot analysis of various cell types using HDAC2 Antibody.



Confocal immunofluorescent analysis of COS cells using HDAC2 Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).



Revision 3



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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IF-IC	H M R Mk	Endogenous	49	Mouse IgG2a	#O15379	8841

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (Immunocytochemistry)	1:50 - 1:200

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

HDAC3 (7G6C5) Mouse mAb detects endogenous levels of total HDAC3 protein. The antibody does not cross-react with other HDAC proteins.

Source / Purification

Monoclonal antibody is produced by immunizing animals with recombinant human HDAC3 protein. The epitope corresponds to a region surrounding Asp415 of human HDAC3.

Background

Acetylation of the histone tail causes chromatin to adopt an "open" conformation, allowing increased accessibility of transcription factors to DNA. The identification of histone acetyltransferases (HATs) and their large multiprotein complexes has yielded important insights into how these enzymes regulate transcription (1,2). HAT complexes interact with sequence-specific activator proteins to target specific genes. In addition to histones, HATs can acetylate nonhistone proteins, suggesting multiple roles for these enzymes (3). In contrast, histone deacetylation promotes a "closed" chromatin conformation and typically leads to repression of gene activity (4). Mammalian histone deacetylases can be divided into three classes on the basis of their similarity to various yeast deacetylases (5). Class I proteins (HDACs 1, 2, 3, and 8) are related to the yeast Rpd3-like proteins, those in class II (HDACs 4, 5, 6, 7, 9, and 10) are related to yeast Hda1-like proteins, and class III proteins are related to the yeast protein Sir2. Inhibitors of HDAC activity are now being explored as potential therapeutic cancer agents (6,7). HDAC3 is a nuclear and cytoplasmic protein that deacetylates both histone (H2A, H3, H4) and non-histone substrates (RelA, SRY, p53, MEF2, PCAF and p300/CBP) (8). HDAC3 deacetylase activity is stimulated by interactions with the N-CoR and SMRT co-repressor proteins. Together, these three proteins form a functional complex that represses transcription associated with nuclear hormone receptors and other transcription factors, including Rev-Erb, COUP-TF, DAX1, MAD and Pit-1 (8,9). Phosphorylation of HDAC3 on Ser424 by casein kinase 2 (CK2) also increases HDAC3 deacetylase activity (9). Subsequently, de-phosphorylation by protein phosphatase 4 (PP4) decreases HDAC3 activity (9).

Background References

1. Marmorstein, R. (2001) *Cell Mol Life Sci* 58, 693-703.
2. Gregory, P.D. et al. (2001) *Exp Cell Res* 265, 195-202.
3. Liu, Y. et al. (2000) *Mol Cell Biol* 20, 5540-53.
4. Cress, W.D. and Seto, E. (2000) *J Cell Physiol* 184, 1-16.
5. Gray, S.G. and Ekström, T.J. (2001) *Exp Cell Res* 262, 75-83.
6. Thiagalingam, S. et al. (2003) *Ann. N.Y. Acad. Sci.* 983, 84-100.
7. Vigushin, D.M. and Coombes, R.C. (2004) *Curr Cancer Drug Targets* 4, 205-18.
8. Karagianni, P. and Wong, J. (2007) *Oncogene* 26, 5439-5449.
9. Zhang, X. et al. (2005) *Genes Dev.* 19, 827-839.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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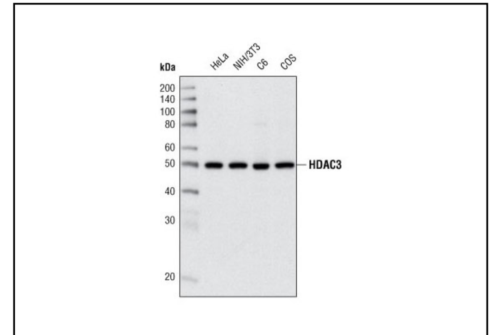
Revision 3

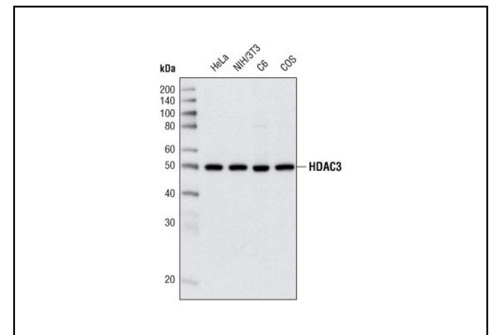
#3949

HDAC3 (7G6C5) Mouse mAb

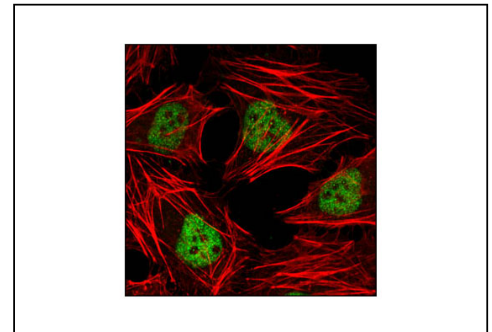
Western blot analysis of extracts from various cell lines using HDAC3 (7G6C5) Mouse mAb.



Western blot analysis of extracts from various cell lines using HDAC3 (7G6C5) Mouse mAb.



Confocal immunofluorescent analysis of HeLa cells using HDAC3 (7G6C5) Mouse mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red).



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Revision 3

#15164 Store at -20C

HDAC4 (D8T3Q) Rabbit mAb

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Support: 877-678-TECH (8324)

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP	H M R Mk	Endogenous	140	Rabbit IgG	#P56524	9759

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

HDAC4 (D8T3Q) Rabbit mAb recognizes endogenous levels of total HDAC4 protein. This antibody does not cross-react with other HDAC proteins, including HDAC5 and HDAC7.

Source / Purification

Monoclonal antibody is produced by immunizing animals with recombinant protein specific to the amino terminus of human HDAC4 protein.

Background

Acetylation of the histone tail causes chromatin to adopt an "open" conformation, allowing increased accessibility of transcription factors to DNA. The identification of histone acetyltransferases (HATs) and their large multiprotein complexes has yielded important insights into how these enzymes regulate transcription (1,2). HAT complexes interact with sequence-specific activator proteins to target specific genes. In addition to histones, HATs can acetylate nonhistone proteins, suggesting multiple roles for these enzymes (3). In contrast, histone deacetylation promotes a "closed" chromatin conformation and typically leads to repression of gene activity (4). Mammalian histone deacetylases can be divided into three classes on the basis of their similarity to various yeast deacetylases (5). Class I proteins (HDACs 1, 2, 3, and 8) are related to the yeast Rpd3-like proteins, those in class II (HDACs 4, 5, 6, 7, 9, and 10) are related to yeast Hda1-like proteins, and class III proteins are related to the yeast protein Sir2. Inhibitors of HDAC activity are now being explored as potential therapeutic cancer agents (6,7).

Background References

1. Marmorstein, R. (2001) *Cell Mol Life Sci* 58, 693-703.
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5. Gray, S.G. and Ekström, T.J. (2001) *Exp Cell Res* 262, 75-83.
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7. Vigushin, D.M. and Coombes, R.C. (2004) *Curr Cancer Drug Targets* 4, 205-18.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

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Applications Key

WB: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
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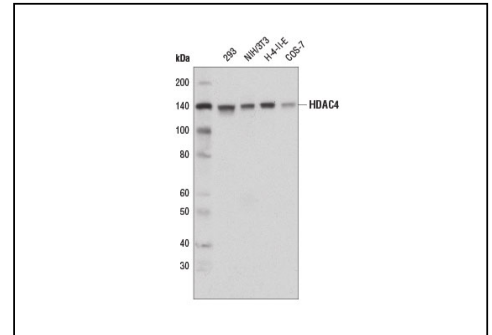
Revision 3

#15164

HDAC4 (D8T3Q) Rabbit mAb

Western blot analysis of extracts from various cell lines using HDAC4 (D8T3Q) Rabbit mAb.



Revision 1

#10831 Store at -20C

HDAC7 (E7O8V) Rabbit mAb

Orders: 877-616-CELL (2355) orders@cellsignal.com

Support: 877-678-TECH (8324)

Web: info@cellsignal.com cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IF-IC	H Mk	Endogenous	124	Rabbit IgG	#Q8WUI4	51564

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (Immunocytochemistry)	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

HDAC7 (E7O8V) Rabbit mAb recognizes endogenous levels of total HDAC7 protein. This antibody does not cross-react with other HDAC proteins, including HDAC4 and HDAC5.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Gly60 of human HDAC7 protein.

Background

Acetylation of the histone tail causes chromatin to adopt an "open" conformation, allowing increased accessibility of transcription factors to DNA. The identification of histone acetyltransferases (HATs) and their large multiprotein complexes has yielded important insights into how these enzymes regulate transcription (1,2). HAT complexes interact with sequence-specific activator proteins to target specific genes. In addition to histones, HATs can acetylate nonhistone proteins, suggesting multiple roles for these enzymes (3). In contrast, histone deacetylation promotes a "closed" chromatin conformation and typically leads to repression of gene activity (4). Mammalian histone deacetylases can be divided into three classes on the basis of their similarity to various yeast deacetylases (5). Class I proteins (HDACs 1, 2, 3, and 8) are related to the yeast Rpd3-like proteins, those in class II (HDACs 4, 5, 6, 7, 9, and 10) are related to yeast Hda1-like proteins, and class III proteins are related to the yeast protein Sir2. Inhibitors of HDAC activity are now being explored as potential therapeutic cancer agents (6,7).

Background References

1. Marmorstein, R. (2001) *Cell Mol Life Sci* 58, 693-703.
2. Gregory, P.D. et al. (2001) *Exp Cell Res* 265, 195-202.
3. Liu, Y. et al. (2000) *Mol Cell Biol* 20, 5540-53.
4. Cress, W.D. and Seto, E. (2000) *J Cell Physiol* 184, 1-16.
5. Gray, S.G. and Ekström, T.J. (2001) *Exp Cell Res* 262, 75-83.
6. Thiagalingam, S. et al. (2003) *Ann. N.Y. Acad. Sci.* 983, 84-100.
7. Vigushin, D.M. and Coombes, R.C. (2004) *Curr Cancer Drug Targets* 4, 205-18.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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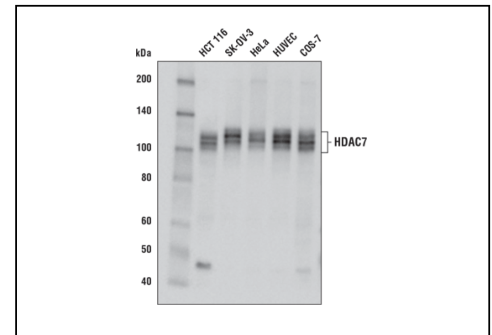
Revision 1

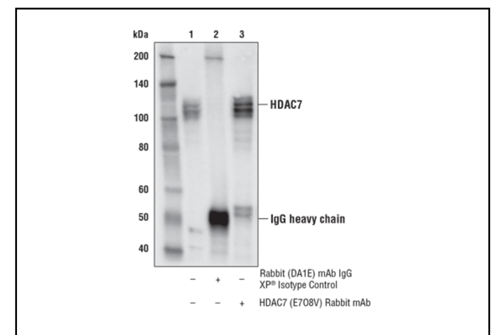
#10831

HDAC7 (E7O8V) Rabbit mAb

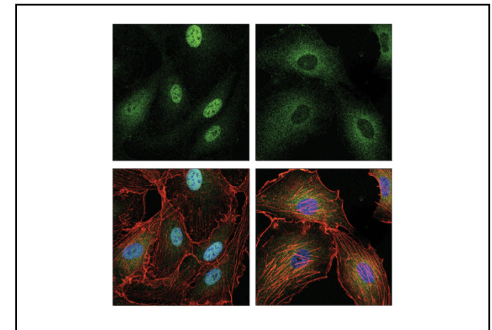
Cell Signaling TECHNOLOGY®



Western blot analysis of extracts from various cell lines using HDAC7 (E7O8V) Rabbit mAb.



Immunoprecipitation of HDAC7 from HCT 116 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is HDAC7 (E7O8V) Rabbit mAb. Western blot analysis was performed using HDAC7 (E7O8V) Rabbit mAb.



Confocal immunofluorescent analysis of HUVEC cells, untreated (left) or treated with TPA #4174 (10 ng/mL, 4 hr; right) to promote cytoplasmic accumulation, using HDAC7 (E7O8V) Rabbit mAb (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Cells were mounted in ProLong® Gold Antifade Reagent with DAPI #8961 (blue).

#8173 Store at -20C

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb

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Support: 877-678-TECH (8324)

Web: info@cellsignal.com cellsignal.com

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IF-IC, FC-FP, ChIP, ChIP-seq, C&R, C&T	H M R Mk	Endogenous	17	Rabbit IgG	#P68431	8350

Product Usage Information

For optimal ChIP and ChIP-seq results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

The CUT&Tag dilution was determined using CUT&Tag Assay Kit #77552.

Application	Dilution
Western Blotting	1:1000
Immunofluorescence (Immunocytochemistry)	1:50 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:50 - 1:200
Chromatin IP	1:100
Chromatin IP-seq	1:100
CUT&RUN	1:100
CUT&Tag	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #87261.

Specificity / Sensitivity

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb recognizes endogenous levels of histone H3 protein only when acetylated at Lys27. This antibody does not cross react with histone H3 acetylated at Lys9, 14, 18, 23, or 56. This antibody shows some cross-reactivity with acetyl-histone H2B lysine 5.

Species predicted to react based on 100% sequence homology

Hamster, Xenopus, Zebrafish, Horse, Guinea Pig, Rabbit

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding acetylated Lys27 of human histone H3 protein.

Background

The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (1,2). Histone acetylation occurs mainly on the amino-terminal tail domains of histones H2A (Lys5), H2B (Lys5, 12, 15, and 20), H3 (Lys9, 14, 18, 23, 27, 36, and 56), and H4 (Lys5, 8, 12, and 16) and is important for the regulation of histone deposition, transcriptional activation, DNA replication, recombination, and DNA repair (1-3). Hyper-acetylation of the histone tails neutralizes the positive charge of these domains and is believed to weaken histone-DNA and nucleosome-nucleosome interactions, thereby destabilizing chromatin structure and increasing the accessibility of DNA to various DNA-binding proteins (4,5). In addition, acetylation of specific lysine residues creates docking sites for a protein module called the bromodomain, which binds to acetylated lysine residues (6). Many transcription and chromatin regulatory proteins contain bromodomains and may be recruited to gene promoters, in part, through binding of acetylated histone tails. Histone acetylation is mediated by histone acetyltransferases (HATs), such as CBP/p300, GCN5L2, PCAF, and Tip60, which are recruited to genes by DNA-bound protein factors to facilitate transcriptional activation (3). Deacetylation, which is mediated by histone deacetylases (HDAC and sirtuin proteins), reverses the effects of acetylation and generally facilitates transcriptional repression (7,8).

Background References

1. Peterson, C.L. and Laniel, M.A. (2004) *Curr Biol* 14, R546-51.
2. Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.

3. Roth, S.Y. et al. (2001) *Annu Rev Biochem* 70, 81-120.
4. Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.
5. Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-41.
6. Yang, X.J. (2004) *Bioessays* 26, 1076-87.
7. Haberland, M. et al. (2009) *Nat Rev Genet* 10, 32-42.
8. Haigis, M.C. and Sinclair, D.A. (2010) *Annu Rev Pathol* 5, 253-95.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IF-IC:** Immunofluorescence (Immunocytochemistry)
FC-FP: Flow Cytometry (Fixed/Permeabilized) **ChIP:** Chromatin IP **ChIP-seq:** Chromatin IP-seq
C&R: CUT&RUN **C&T:** CUT&Tag

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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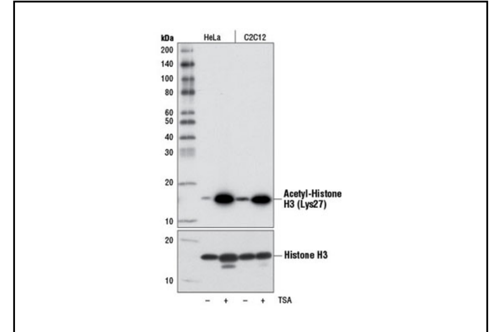
Revision 9

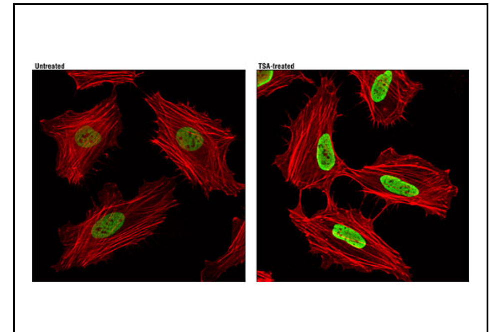
#8173

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb

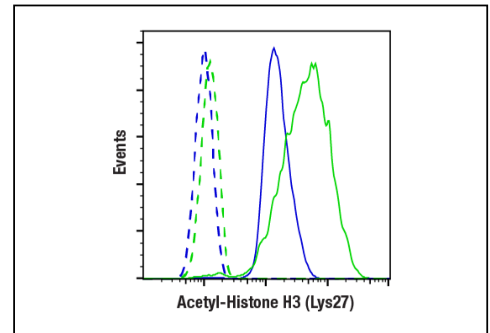
Western blot analysis of extracts from HeLa and C2C12 cells, untreated (-) or treated (+) with Trichostatin A (TSA) #9950 (1 µM, 18 hr), using Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb (upper) or Histone H3 (D1H2) XP® Rabbit mAb #4499 (lower).



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or treated with Trichostatin A (TSA) #9950 (1 uM, 4 hr; right), using Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).



Flow cytometric analysis of HeLa cells, untreated (blue) or treated with Trichostatin A (TSA) #9950 (1 µM, 18 hr; green) using Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.



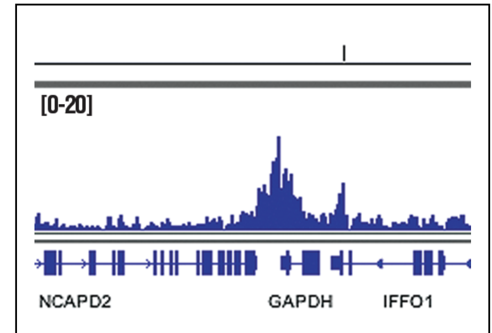
Revision 9

#8173

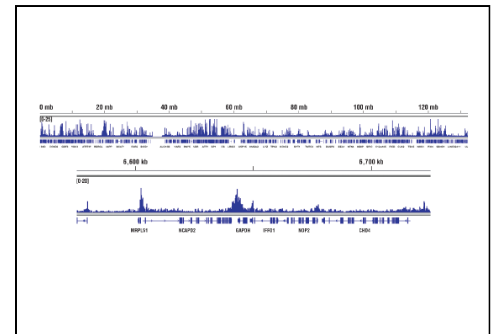
Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

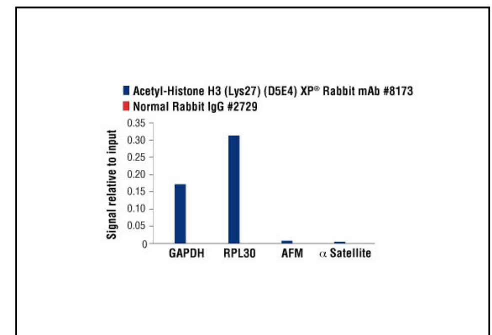
Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figure shows binding across GAPDH, a known target gene of H3K27Ac (see additional figure containing ChIP-qPCR data).



Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figure shows binding across chromosome 12 (upper), including GAPDH (lower), a known target gene of H3K27Ac (see additional figure containing ChIP-qPCR data).



Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells and either Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human GAPDH Exon 1 Primers #5516, SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human AFM Intron 1 Primers #5098, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

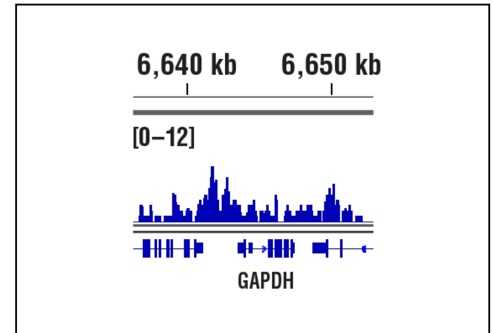


Revision 9

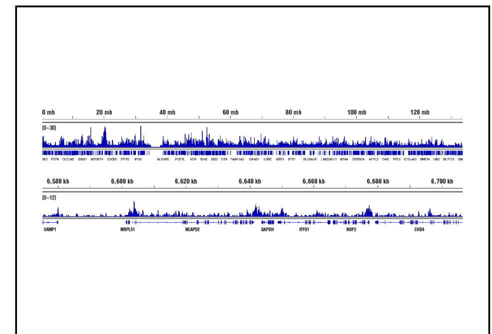
#8173

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb

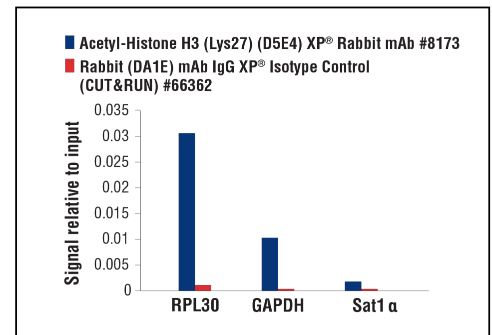
Cell Signaling TECHNOLOGY®



CUT&RUN was performed with HeLa cells and Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb, using CUT&RUN Assay Kit #86652. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figures show binding across GAPDH, a known target gene of H3K27Ac (see additional figure containing CUT&RUN-qPCR data).



CUT&RUN was performed with HeLa cells and Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb, using CUT&RUN Assay Kit #86652. DNA Libraries were prepared using DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795. The figures show binding across chromosome 12 (upper), including GAPDH (lower), a known target gene of H3K27Ac (see additional figure containing CUT&RUN-qPCR data).



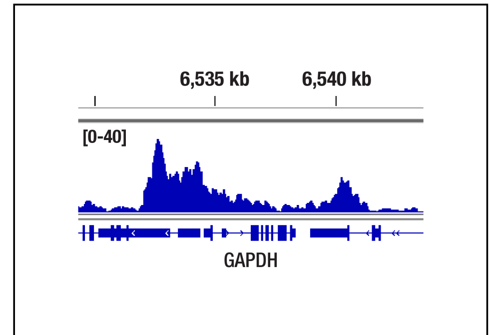
CUT&RUN was performed with HeLa cells and either Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb or Rabbit (DA1E) mAb IgG XP® Isotype Control (CUT&RUN) #66362, using CUT&RUN Assay Kit #86652. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human GAPDH Exon 1 Primers #5516, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Revision 9

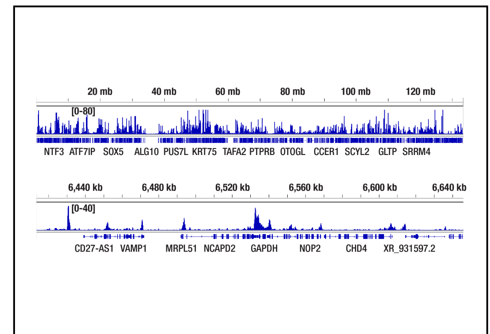
#8173

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb

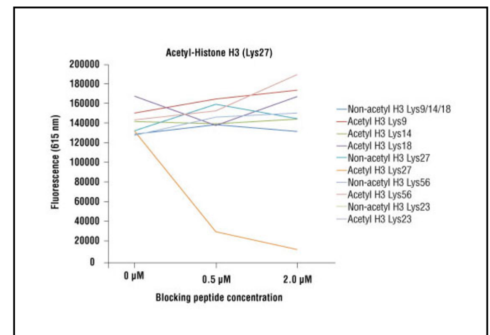
CUT&Tag was performed with HeLa cells and Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb, using CUT&Tag Assay Kit #77552. DNA library was prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. The figure shows binding across GAPDH, a known target gene of H3K27ac (see our ChIP-qPCR figure).



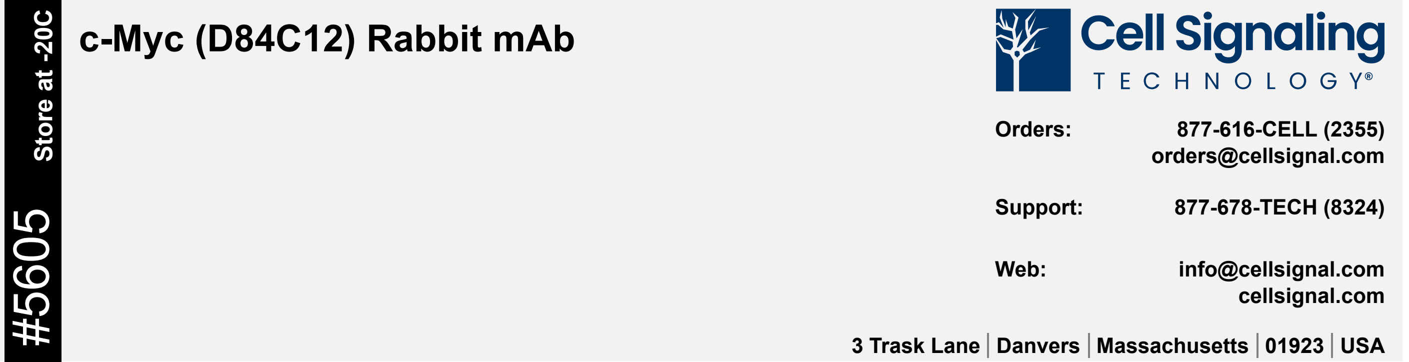
CUT&Tag was performed with HeLa cells and Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb, using CUT&Tag Assay Kit #77552. DNA library was prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. The figures show binding across chromosome 12 (upper), including GAPDH (lower), a known target gene of H3K27ac (see our ChIP-qPCR figure).



Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb specificity was determined by peptide ELISA. The graph depicts the binding of the antibody to precoated acetyl-histone H3 (Lys27) peptide in the presence of increasing concentrations of various competitor peptides. As shown, only the acetyl-histone H3 (Lys27) peptide competed away binding of the antibody.



Revision 6



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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IF-IC	H M R	Endogenous	57-65	Rabbit IgG	#P01106	4609

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:50 - 1:250
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

c-Myc (D84C12) Rabbit mAb detects endogenous levels of total c-Myc protein. This antibody is not recommended for detection of Myc-tagged fusion proteins (use Cell Signaling Technology cat. #2276 or #2278).

Species predicted to react based on 100% sequence homology

Dog, Pig

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues of c-Myc.

Background

Members of the Myc/Max/Mad network function as transcriptional regulators with roles in various aspects of cell behavior, including proliferation, differentiation, and apoptosis (1). These proteins share a common basic-helix-loop-helix leucine zipper (bHLH-ZIP) motif required for dimerization and DNA-binding. Max was originally discovered based on its ability to associate with c-Myc and found to be required for the ability of Myc to bind DNA and activate transcription (2). Subsequently, Max has been viewed as a central component of the transcriptional network, forming homodimers as well as heterodimers with other members of the Myc and Mad families (1). The association between Max and either Myc or Mad can have opposing effects on transcriptional regulation and cell behavior (1). The Mad family consists of four related proteins; Mad1, Mad2 (Mxi1), Mad3, and Mad4, and the more distantly related members of the bHLH-ZIP family, Mnt and Mga. Like Myc, the Mad proteins are tightly regulated with short half-lives. In general, Mad family members interfere with Myc-mediated processes, such as proliferation, transformation, and prevention of apoptosis by inhibiting transcription (3,4).

Background References

1. Baudino, T.A. and Cleveland, J.L. (2001) *Mol Cell Biol* 21, 691-702.
2. Blackwood, E.M. and Eisenman, R.N. (1991) *Science* 251, 1211-7.
3. Henriksson, M. and Lüscher, B. (1996) *Adv Cancer Res* 68, 109-82.
4. Grandori, C. et al. (2000) *Annu Rev Cell Dev Biol* 16, 653-99.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **W-S:** Simple Western™ **IF-IC:** Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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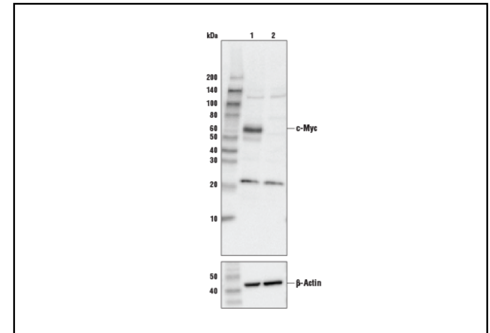
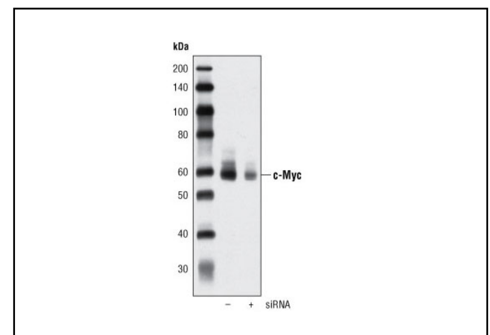
Revision 6

#5605

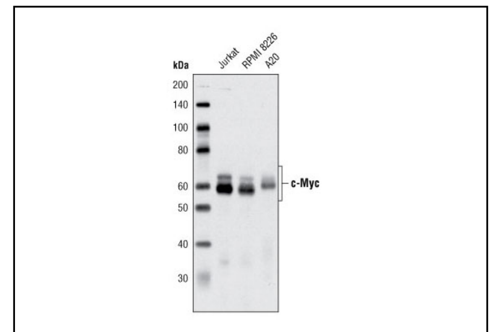
c-Myc (D84C12) Rabbit mAb



Western blot analysis of extracts from control HEK293 cells (lane 1) or c-Myc knockout HEK293 cells (lane 2) using c-Myc (D84C12) Rabbit mAb Antibody, #5605 (upper) or β-actin (13E5) Rabbit mAb, #4970 (lower). The absence of signal in the c-Myc knockout HEK293 cells confirms specificity of the antibody for c-Myc.



Western blot analysis of extracts from HeLa cells, mock transfected or transfected with SignalSilence® c-Myc siRNA I #6341, using c-Myc (D84C12) Rabbit mAb.



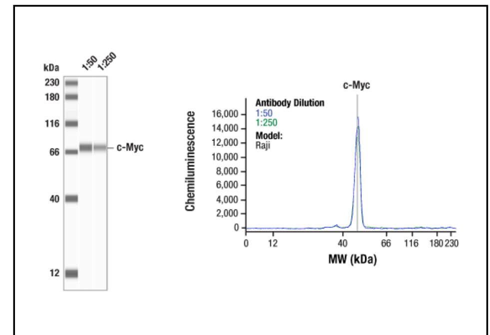
Western blot analysis of extracts from various cell lines using c-Myc (D84C12) Rabbit mAb.

Revision 6

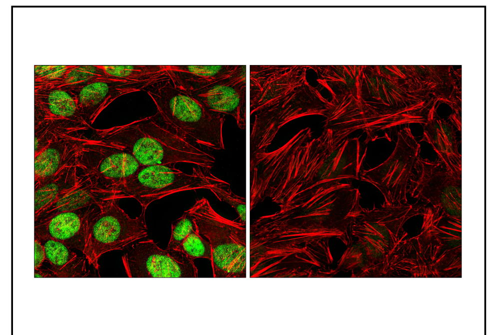
#5605

c-Myc (D84C12) Rabbit mAb

Simple Western™ analysis of lysates (1 mg/mL) from Raji cells using c-Myc (D84C12) Rabbit mAb #5605. The virtual lane view (left) shows a single target band (as indicated) at 1:50 and 1:250 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:50 (blue line) and 1:250 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



Confocal immunofluorescent analysis of HeLa cells, mock-transfected (left) or transfected with SignalSilence® c-Myc siRNA I #6341 (right), using c-Myc (D84C12) Rabbit mAb (green). Actin filaments have been labeled wth DY-554 phalloidin (red).



Revision 12

#9661 Store at -20C

Cleaved Caspase-3 (Asp175) Antibody

Orders: 877-616-CELL (2355) orders@cellsignal.com

Support: 877-678-TECH (8324)

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IP, IHC-P, IF-IC, FC-FP	H M R Mk	Endogenous	17, 19	Rabbit	#P42574	836

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:400
Immunofluorescence (Immunocytochemistry)	1:400
Flow Cytometry (Fixed/Permeabilized)	1:800

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Cleaved Caspase-3 (Asp175) Antibody detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full length caspase-3 or other cleaved caspases. This antibody detects non-specific caspase substrates by western blot. Non-specific labeling may be observed by immunofluorescence in specific sub-types of healthy cells in fixed-frozen tissues (e.g. pancreatic alpha-cells). Nuclear background may be observed in rat and monkey samples.

Species predicted to react based on 100% sequence homology

Bovine, Dog, Pig

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues adjacent to (Asp175) in human caspase-3.

Background

Caspase-3 (CPP-32, Apopain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires the aspartic acid residue at the P1 position (2).

Background References

1. Fernandes-Alnemri, T. et al. (1994) *J Biol Chem* 269, 30761-4.
2. Nicholson, D.W. et al. (1995) *Nature* 376, 37-43.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation
IHC-P: Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry)
FC-FP: Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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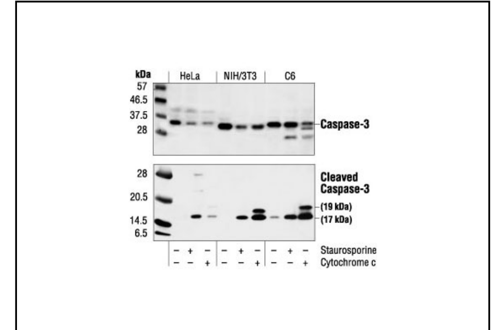
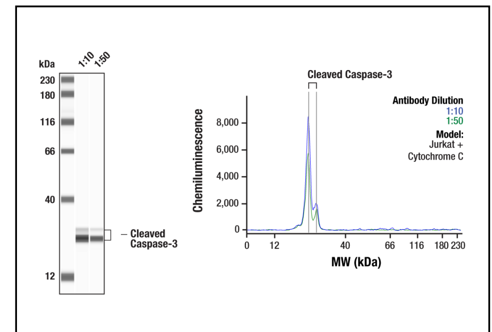
Revision 12

#9661

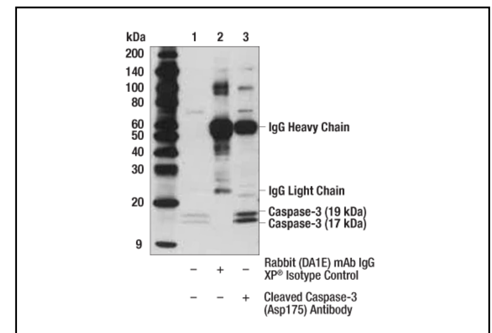
Cleaved Caspase-3 (Asp175) Antibody



Western blot analysis of extracts from HeLa, NIH/3T3 and C6 cells untreated, staurosporine-treated (3hrs, 1 µM in vivo) or cytochrome c-treated (1hr, 0.25 mg/ml in vitro), using Caspase-3 Antibody #9662 (upper) or Cleaved Caspase-3 (Asp175) Antibody (lower).



Simple Western™ analysis of lysates (0.1 mg/mL) from Jurkat cells treated with Cytochrome C using Cleaved Caspase-3 (Asp175) Antibody #9661. The virtual lane view (left) shows two target bands (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



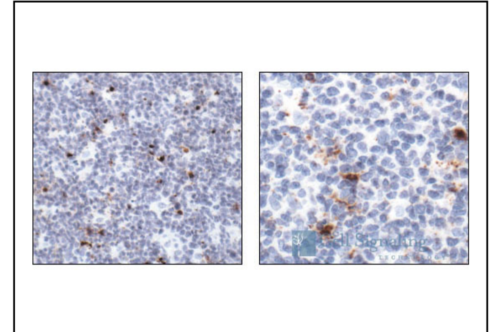
Immunoprecipitation of cleaved caspase-3 from Jurkat extracts treated with Etoposide #2200 (25 mM; 5 hr). Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is Cleaved Caspase-3 (Asp175) Antibody. Western blot analysis was performed using Cleaved Caspase-3 (Asp175) Antibody. Anti-rabbit IgG, HRP-linked Antibody #7074 was used as a secondary antibody.

Revision 12

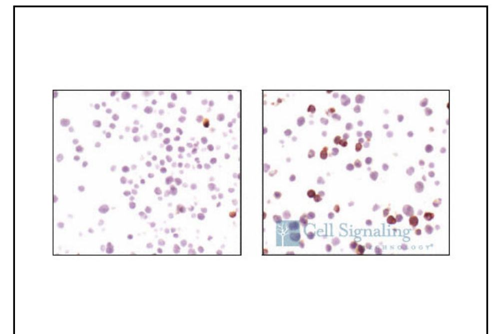
#9661

Cleaved Caspase-3 (Asp175) Antibody

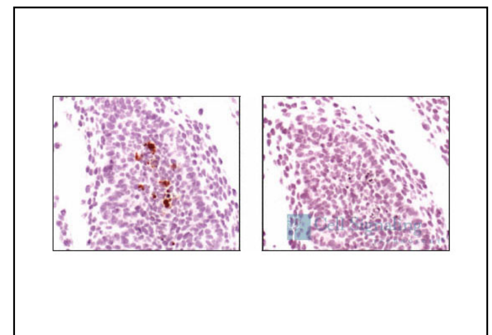
Immunohistochemical analysis of paraffin-embedded human tonsil, showing cytoplasmic and perinuclear localization in apoptotic cells (low and high magnification), using Cleaved Caspase-3 (Asp175) Antibody.



Immunohistochemical analysis using Cleaved caspase-3 (Asp175) antibody on SignalSlide™ Cleaved Caspase-3 IHC controls #8104 (paraffin-embedded Jurkat cells, untreated (left) or etoposide-treated (right)).



Immunohistochemical analysis of paraffin-embedded mouse embryo, using Cleaved Caspase-3 (Asp175) Antibody preincubated with control peptide (left) or Cleaved Caspase-3 (Asp175) Blocking Peptide #1050 (right).



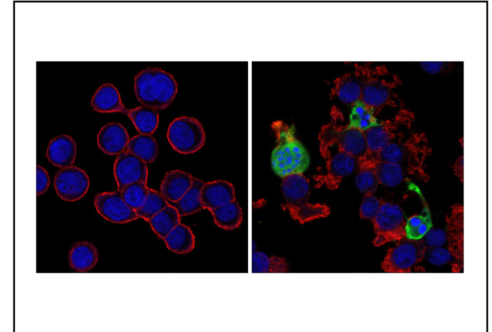
Revision 12

#9661

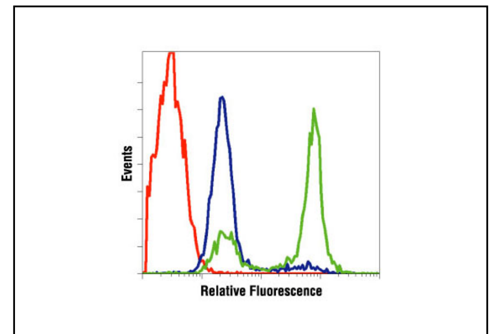
Cleaved Caspase-3 (Asp175) Antibody

Cell Signaling TECHNOLOGY®

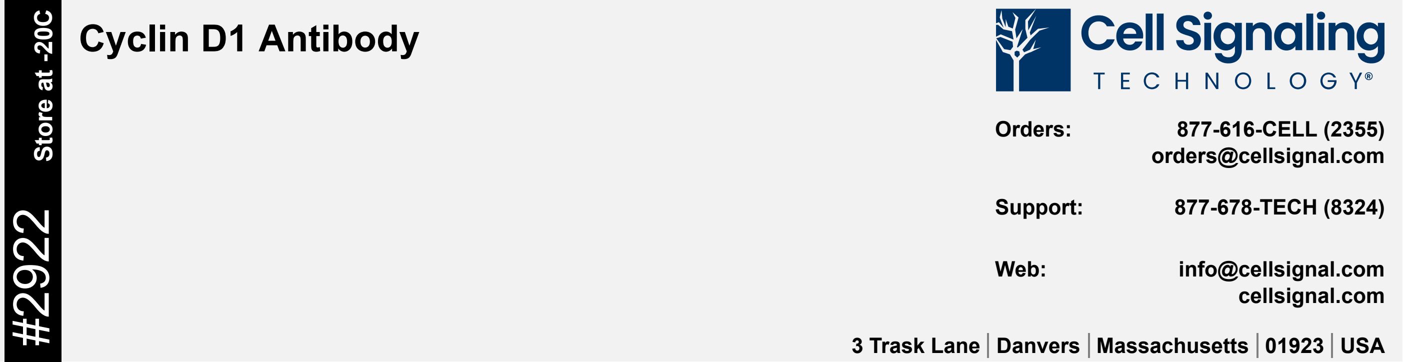
Confocal immunofluorescent images of HT-29 cells, untreated (left) or Staurosporine #9953 treated (right), labeled with Cleaved Caspase-3 (Asp175) Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin #8953 (red). Blue pseudocolor = DRAQ5® (fluorescent DNA dye).



Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with etoposide #2200 (green), using Cleaved Caspase-3 (Asp175) Antibody compared to a nonspecific negative control antibody (red).



Revision 5



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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, IP	H	Endogenous	36	Rabbit	#P24385	595

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Cyclin D1 Antibody detects endogenous levels of cyclin D1. It does not cross-react with other family members.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu259 of human cyclin D1 protein. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Activity of the cyclin-dependent kinases CDK4 and CDK6 is regulated by T-loop phosphorylation, by the abundance of their cyclin partners (the D-type cyclins), and by association with CDK inhibitors of the Cip/Kip or INK family of proteins (1). The inactive ternary complex of cyclin D/CDK4 and p27 Kip1 requires extracellular mitogenic stimuli for the release and degradation of p27 concomitant with a rise in cyclin D levels to affect progression through the restriction point and Rb-dependent entry into S-phase (2). The active complex of cyclin D/CDK4 targets the retinoblastoma protein for phosphorylation, allowing the release of E2F transcription factors that activate G1/S-phase gene expression (3). Levels of cyclin D protein drop upon withdrawal of growth factors through downregulation of protein expression and phosphorylation-dependent degradation (4).

Background References

1. Hirai, H. et al. (1995) *Mol Cell Biol* 15, 2672-81.
2. Sherr, C.J. (1996) *Science* 274, 1672-7.
3. Lukas, J. et al. (1996) *Mol Cell Biol* 16, 6917-25.
4. Diehl, J.A. et al. (1997) *Genes Dev* 11, 957-72.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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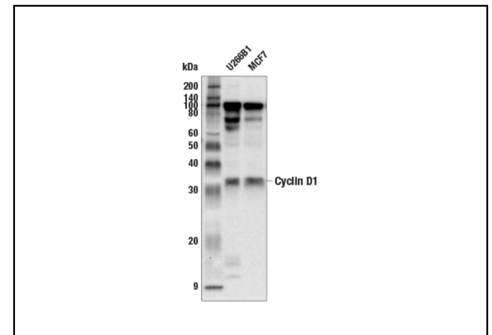
Revision 5

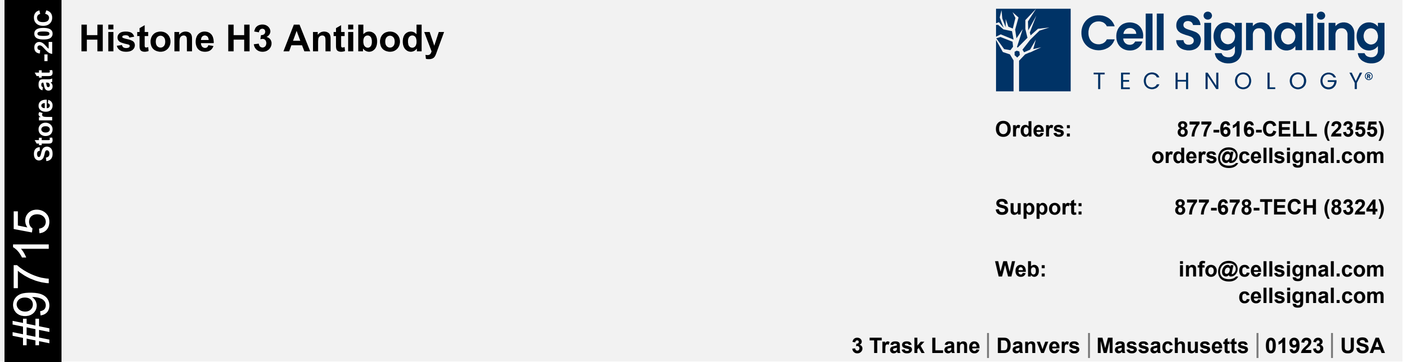
#2922

Cyclin D1 Antibody

Western blot analysis of extracts from U266B1 and MCF7 cells, using Cyclin D1 Antibody.



Revision 1



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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB	H M R Mk Z B Pg	Endogenous	17	Rabbit	#P68431	8350

Product Usage Information

Application	Dilution
Western Blotting	1:1000

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Histone H3 Antibody detects endogenous levels of total histone H3 protein. This antibody does not cross-react with other histones.

Species predicted to react based on 100% sequence homology

D. melanogaster

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy-terminal sequence of human histone H3. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various posttranslational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15, and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28, and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation at Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation at Thr3 of H3 in prophase and its dephosphorylation during anaphase (11).

Background References

1. Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.
2. Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637-41.
3. Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41-5.
4. Cheung, P. et al. (2000) *Cell* 103, 263-71.
5. Bernstein, B.E. and Schreiber, S.L. (2002) *Chem Biol* 9, 1167-73.
6. Jaskelioff, M. and Peterson, C.L. (2003) *Nat Cell Biol* 5, 395-9.
7. Thorne, A.W. et al. (1990) *Eur J Biochem* 193, 701-13.
8. Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348-60.
9. Goto, H. et al. (1999) *J Biol Chem* 274, 25543-9.
10. Preuss, U. et al. (2003) *Nucleic Acids Res* 31, 878-85.
11. Dai, J. et al. (2005) *Genes Dev* 19, 472-88.
12. Hoover, L.L. et al. (2008) *Biochim Biophys Acta* 1783, 2279-86.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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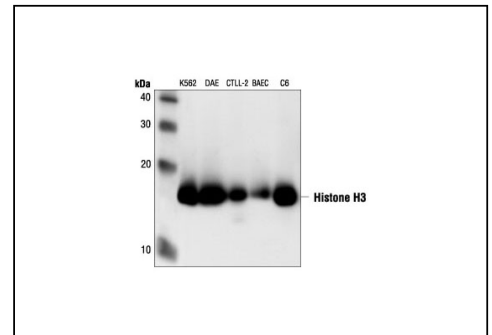
Revision 1

#9715

Histone H3 Antibody

Western blot analysis of extracts from various cell lines using Histone H3 Antibody.



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Revision 5

#2577 Store at -20C

Phospho-Histone H2A.X (Ser139) Antibody

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IF-IC, FC-FP	H M R Mk	Endogenous	15	Rabbit	#P16104	3014

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:50 - 1:250
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600
Flow Cytometry (Fixed/Permeabilized)	1:200

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Phospho-H2A.X (Ser139) Antibody detects endogenous levels of H2A.X only when phosphorylated at Ser139.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser139 of human H2A.X. Antibodies are purified by protein A and peptide affinity chromatography.

Background

Histone H2A.X is a variant histone that represents approximately 10% of the total H2A histone proteins in normal human fibroblasts (1). H2A.X is required for checkpoint-mediated cell cycle arrest and DNA repair following double-stranded DNA breaks (1). DNA damage, caused by ionizing radiation, UV-light, or radiomimetic agents, results in rapid phosphorylation of H2A.X at Ser139 by PI3K-like kinases, including ATM, ATR, and DNA-PK (2,3). Within minutes following DNA damage, H2A.X is phosphorylated at Ser139 at sites of DNA damage (4). This very early event in the DNA-damage response is required for recruitment of a multitude of DNA-damage response proteins, including MDC1, NBS1, RAD50, MRE11, 53BP1, and BRCA1 (1). In addition to its role in DNA-damage repair, H2A.X is required for DNA fragmentation during apoptosis and is phosphorylated by various kinases in response to apoptotic signals. H2A.X is phosphorylated at Ser139 by DNA-PK in response to cell death receptor activation, c-Jun N-terminal Kinase (JNK1) in response to UV-A irradiation, and p38 MAPK in response to serum starvation (5-8). H2A.X is constitutively phosphorylated on Tyr142 in undamaged cells by WSTF (Williams-Beuren syndrome transcription factor) (9,10). Upon DNA damage, and concurrent with phosphorylation of Ser139, Tyr142 is dephosphorylated at sites of DNA damage by recruited EYA1 and EYA3 phosphatases (9). While phosphorylation at Ser139 facilitates the recruitment of DNA repair proteins and apoptotic proteins to sites of DNA damage, phosphorylation at Tyr142 appears to determine which set of proteins are recruited. Phosphorylation of H2A.X at Tyr142 inhibits the recruitment of DNA repair proteins and promotes binding of pro-apoptotic factors such as JNK1 (9). Mouse embryonic fibroblasts expressing only mutant H2A.X Y142F, which favors recruitment of DNA repair proteins over apoptotic proteins, show a reduced apoptotic response to ionizing radiation (9). Thus, it appears that the balance of H2A.X Tyr142 phosphorylation and dephosphorylation provides a switch mechanism to determine cell fate after DNA damage.

Background References

1. Yuan, J. et al. (2010) *FEBS Lett* 584, 3717-24.
2. Rogakou, E.P. et al. (1998) *J Biol Chem* 273, 5858-68.
3. Burma, S. et al. (2001) *J Biol Chem* 276, 42462-7.
4. Rogakou, E.P. et al. (1999) *J Cell Biol* 146, 905-16.
5. Mukherjee, B. et al. (2006) *DNA Repair (Amst)* 5, 575-90.
6. Solier, S. et al. (2009) *Mol Cell Biol* 29, 68-82.
7. Lu, C. et al. (2006) *Mol Cell* 23, 121-32.
8. Lu, C. et al. (2008) *FEBS Lett* 582, 2703-8.
9. Cook, P.J. et al. (2009) *Nature* 458, 591-6.
10. Xiao, A. et al. (2009) *Nature* 457, 57-62.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **W-S:** Simple Western™ **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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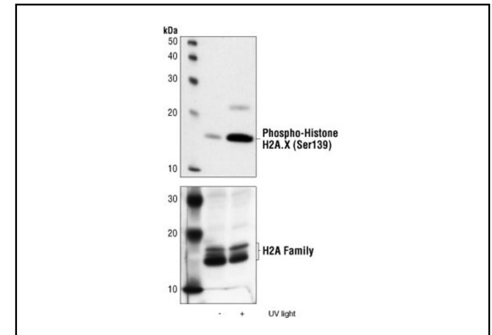
Revision 5

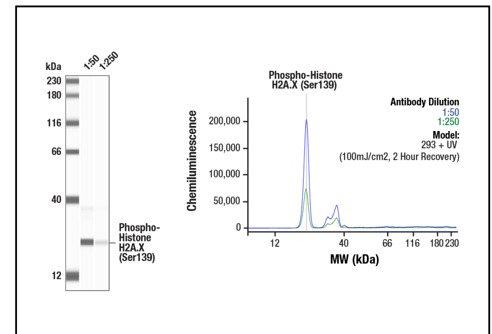
#2577

Phospho-Histone H2A.X (Ser139) Antibody

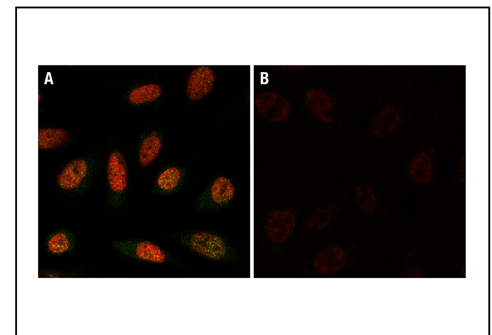
Western blot analysis of extracts from 293 cells, untreated or UV-treated, using Phospho-Histone H2A.X (Ser139) Antibody (upper) or Histone H2A Antibody #2572 (lower).



Simple Western™ analysis of lysates (1.0 mg/mL) from 293 cells treated with UV (100mJ/cm2, 2 Hour Recovery) using Phospho-Histone H2A.X (Ser139) Antibody #2577. The virtual lane view (left) shows the target band (as indicated) at 1:50 and 1:250 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:50 (blue line) and 1:250 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



Confocal microscopic images of HeLa cells, UV treated (A) and untreated (B), showing nuclear stain with Phospho-Histone H2A.X (Ser139) Antibody (red) and Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb #9255 (green).

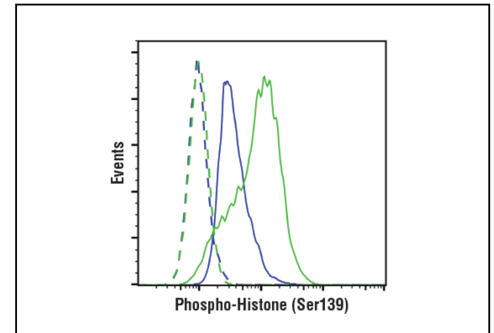


Revision 5

#2577

Phospho-Histone H2A.X (Ser139) Antibody

Flow cytometric analysis of HeLa cells, untreated (blue) or treated with UV (100mJ/cm2, 2 hr recovery; green) using Phospho-Histone H2A.X (Ser139) Antibody (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary control.



Revision 1

#12079 Store at -20C

MCM2 (1E7) Mouse mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IHC-P, IF-IC	H Mk	Endogenous	125	Mouse IgG1	#P49736	4171

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:200
Immunohistochemistry (Paraffin)	1:400
Immunofluorescence (Immunocytochemistry)	1:200

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

MCM2 (1E7) Mouse mAb recognizes endogenous levels of total MCM2 protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with recombinant protein specific to the amino terminus of human MCM2 protein.

Background

The minichromosome maintenance (MCM) 2-7 proteins are a family of six related proteins required for initiation and elongation of DNA replication. MCM2-7 bind together to form the heterohexameric MCM complex that is thought to act as a replicative helicase at the DNA replication fork (1-5). This complex is a key component of the pre-replication complex (pre-RC) (reviewed in 1). Cdc6 and CDT1 recruit the MCM complex to the origin recognition complex (ORC) during late mitosis/early G1 phase forming the pre-RC and licensing the DNA for replication (reviewed in 2). Licensing of the chromatin permits the DNA to replicate only once per cell cycle, thereby helping to ensure that genetic alterations and malignant cell growth do not occur (reviewed in 3). Phosphorylation of the MCM2, MCM3, MCM4, and MCM6 subunits appears to regulate MCM complex activity and the initiation of DNA synthesis (6-8). CDK1 phosphorylation of MCM3 at Ser112 during late mitosis/early G1 phase has been shown to initiate complex formation and chromatin loading *in vitro* (8). Phosphorylation of MCM2 at serine 139 by cdc7/dbf4 coincides with the initiation of DNA replication (9). MCM proteins are removed during DNA replication, causing chromatin to become unlicensed through inhibition of pre-RC reformation. Studies have shown that the MCM complex is involved in checkpoint control by protecting the structure of the replication fork and assisting in restarting replication by recruiting checkpoint proteins after arrest (reviewed in 3,10).

Background References

1. Lei, M. and Tye, B.K. (2001) *J Cell Sci* 114, 1447-54.
2. Lygerou, Z. and Nurse, P. (2000) *Science* 290, 2271-3.
3. Forsburg, S.L. (2004) *Microbiol Mol Biol Rev* 68, 109-31.
4. Tye, B.K. and Sawyer, S. (2000) *J Biol Chem* 275, 34833-6.
5. Labib, K. et al. (2000) *Science* 288, 1643-7.
6. Charych, D.H. et al. (2008) *J Cell Biochem* 104, 1075-86.
7. Masai, H. et al. (2006) *J Biol Chem* 281, 39249-61.
8. Lin, D.I. et al. (2008) *Proc Natl Acad Sci USA* 105, 8079-84.
9. Tsuji, T. et al. (2006) *Mol Biol Cell* 17, 4459-72.
10. Bailis, J.M. et al. (2008) *Mol Cell Biol* 28, 1724-38.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin)
IF-IC: Immunofluorescence (Immunocytochemistry)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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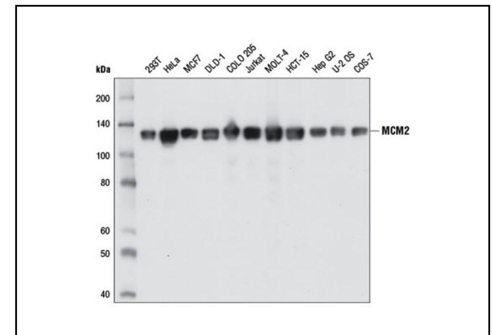
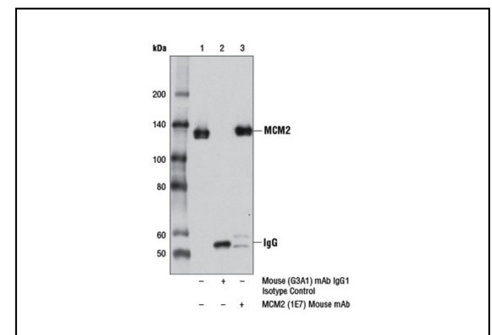
Revision 1

#12079

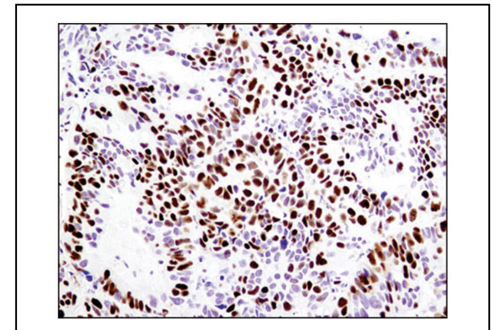
MCM2 (1E7) Mouse mAb



Western blot analysis of extracts from various cell lines using MCM2 (1E7) Mouse mAb.



Immunoprecipitation of MCM2 from Jurkat cell extracts using Mouse (G3A1) mAb IgG1 Isotype Control #5415 (lane 2) or MCM2 (1E7) Mouse mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using MCM2 (1E7) Mouse mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using MCM2 (1E7) Mouse mAb.

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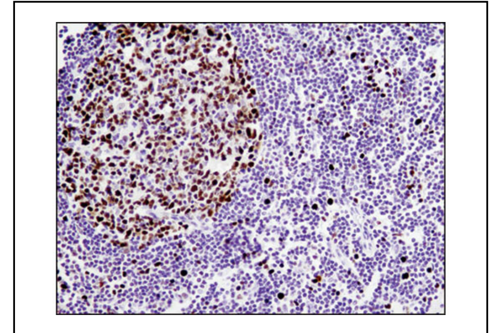
Revision 1

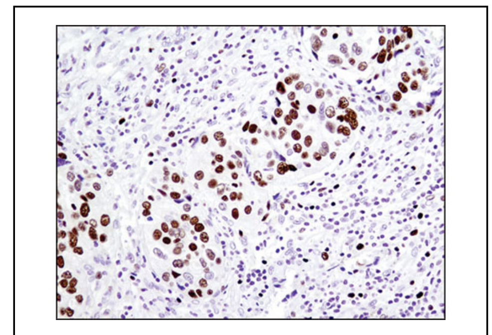
#12079

MCM2 (1E7) Mouse mAb

Immunohistochemical analysis of paraffin-embedded human lymph node using MCM2 (1E7) Mouse mAb.



Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma using MCM2 (1E7) Mouse mAb.



Confocal immunofluorescent analysis of HeLa cells using MCM2 (1E7) Mouse mAb (green) and β-Actin (13E5) Rabbit mAb (Alexa Fluor® 647 Conjugate) #8584.

Revision 3

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source:	UniProt ID:	Entrez-Gene Id:
WB, W-S	H M R Mk	Endogenous	89, 116	Rabbit	#P09874	142

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

PARP Antibody detects endogenous levels of full length PARP1 (116 kDa), as well as the large fragment (89 kDa) of PARP1 resulting from caspase cleavage. The antibody does not cross-react with related proteins or other PARP isoforms.

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the caspase cleavage site in PARP. Antibodies are purified by protein A and peptide affinity chromatography.

Background

PARP, a 116 kDa nuclear poly (ADP-ribose) polymerase, appears to be involved in DNA repair in response to environmental stress (1). This protein can be cleaved by many ICE-like caspases *in vitro* (2,3) and is one of the main cleavage targets of caspase-3 *in vivo* (4,5). In human PARP, the cleavage occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA-binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) (2,4). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (6).

Background References

1. Satoh, M.S. and Lindahl, T. (1992) *Nature* 356, 356-358.
2. Lazebnik, Y. A. et al. (1994) *Nature* 371, 346-347.
3. Cohen, G.M. (1997) *Biochem. J.* 326, 1-16.
4. Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.
5. Tewari, M. et al. (1995) *Cell* 81, 801-809.
6. Oliver, F.J. et al. (1998) *J. Biol. Chem.* 273, 33533-33539.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **W-S:** Simple Western™

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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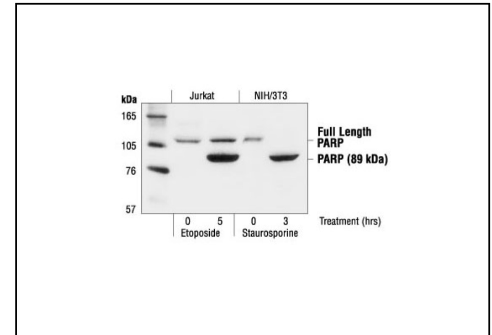
Revision 3

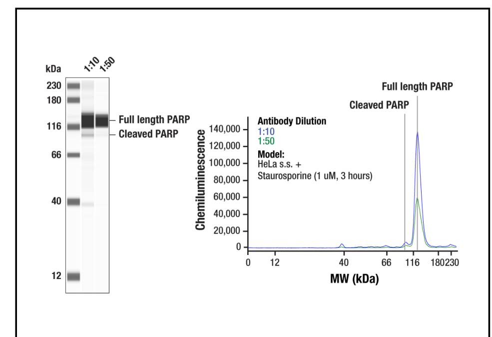
#9542

PARP Antibody

Western blot analysis of extracts from NIH/3T3 cells, untreated or staurosporine-treated (1 μM), and Jurkat cells, untreated or etoposide-treated (25 μM), using PARP Antibody.



Simple Western™ analysis of lysates (1 mg/mL) from serum-starved HeLa cells treated with Staurosporine (1 uM, 3 hours) using PARP Antibody #9542. The virtual lane view (left) shows the target bands (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



Revision 9

#5625 Store at -20C

Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, W-S, IP, IHC-P, IF-IC, FC-FP	H Mk	Endogenous	89	Rabbit IgG	#P09874	142

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:50
Immunofluorescence (Immunocytochemistry)	1:400
Flow Cytometry (Fixed/Permeabilized)	1:200 - 1:800

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier-free (BSA and azide free) version of this product see product #95696.

Specificity / Sensitivity

Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb detects endogenous levels of the large fragment (89 kDa) of human PARP1 protein produced by caspase cleavage. The antibody does not recognize full length PARP1 or other PARP isoforms.

Source / Purification

Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp214 in human PARP.

Background

PARP, a 116 kDa nuclear poly (ADP-ribose) polymerase, appears to be involved in DNA repair in response to environmental stress (1). This protein can be cleaved by many ICE-like caspases *in vitro* (2,3) and is one of the main cleavage targets of caspase-3 *in vivo* (4,5). In human PARP, the cleavage occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA-binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) (2,4). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (6).

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3. Cohen, G.M. (1997) *Biochem. J.* 326, 1-16.
4. Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.
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6. Oliver, F.J. et al. (1998) *J. Biol. Chem.* 273, 33533-33539.

Species Reactivity

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Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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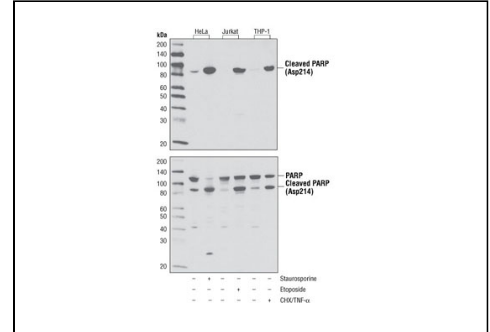
Revision 9

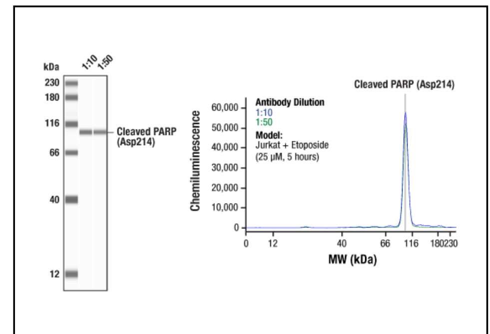
#5625

Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb

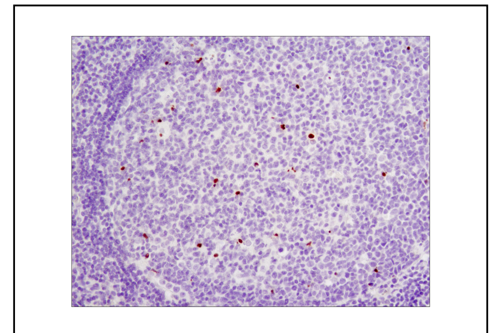
Western blot analysis of extracts from HeLa cells, untreated or treated with Staurosporine #9953 (1 µM, 3 hr), Jurkat cells, untreated or etoposide-treated (25 µM, overnight), and THP-1 cells, untreated or cycloheximide-treated (CHX, 10 µg/ml, overnight) followed by treatment with TNF-α #8902 (20 ng/ml, 4 hr), using Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (upper), or total PARP Antibody #9542 (lower).



Simple Western™ analysis of lysates (1 mg/mL) from Jurkat cells treated with Etoposide (25 µM, 5 hours) using Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb #5625. The virtual lane view (left) shows a single target band (as indicated) at 1:10 and 1:50 dilutions of primary antibody. The corresponding electropherogram view (right) plots chemiluminescence by molecular weight along the capillary at 1:10 (blue line) and 1:50 (green line) dilutions of primary antibody. This experiment was performed under reducing conditions on the Jess™ Simple Western instrument from ProteinSimple, a BioTechne brand, using the 12-230 kDa separation module.



Immunohistochemical analysis of paraffin-embedded human tonsil using Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb.

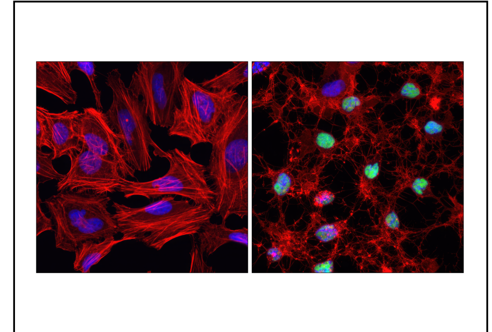


Revision 9

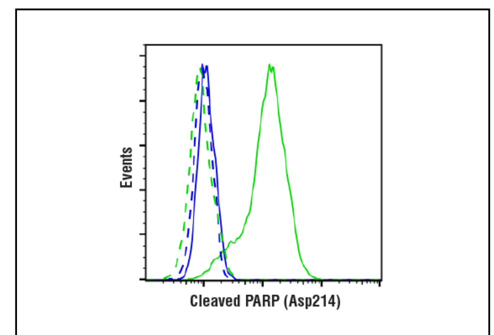
#5625

Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb

Confocal immunofluorescent analysis of HeLa cells, untreated (left) or treated with Staurosporine #9953 (right), using Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (green). Actin filament were labeled with DY-554 phalloidin. Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with Etoposide #2200 (25 uM, 18 hr; green) using Cleaved PARP (Asp214) (D64E10) XP® Rabbit mAb (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.



Revision 1

#13281 Store at -20C

Phospho-Merlin (Ser518) (D5A4I) Rabbit mAb

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP	H M R Mk	Endogenous	70	Rabbit IgG	#P35240	4771

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:50

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

Phospho-Merlin (Ser518) (D5A4I) Rabbit mAb recognizes endogenous levels of merlin protein only when phosphorylated at Ser518.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser518 of human merlin protein.

Background

Neurofibromatosis 2 (NF2) is an autosomal dominant, inherited disorder characterized by the occurrence of vestibular schwannomas, meningiomas, and other nervous system tumors. Both the familial tumors of NF2 and equivalent sporadic tumors found in the general population are caused by inactivation of the *NF2* tumor suppressor gene. Merlin (moesin, ezrin, and radixin-like protein) is the *NF2* gene product, displaying striking similarity to ezrin, radixin, and moesin (ERM) proteins. Regulation of merlin (also called schwannomin) and ERM proteins involves intramolecular and intermolecular head-to-tail associations between family members (1). Merlin and ERM proteins act as linkers between the plasma membrane and the cytoskeleton, affecting cell morphology, polarity, and signal transduction (2). Merlin is phosphorylated by the Rac/Cdc42 effector p21-activated kinase (PAK) at Ser518, negatively regulating Rac (3,4).

Background References

1. Ramesh, V. (2004) *Nat. Rev. Neurosci.* 5, 462-70.
2. Bretscher, A. et al. (2002) *Nat. Rev. Mol. Cell Biol.* 3, 586-99.
3. Xiao, G. H. et al. (2002) *J. Biol. Chem.* 277, 883-6.
4. Kissil, J. L. et al. (2003) *Mol. Cell* 12, 841-9.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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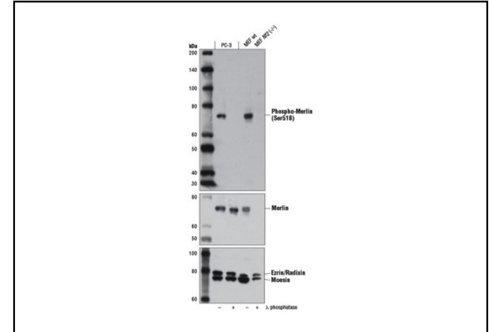
Revision 1

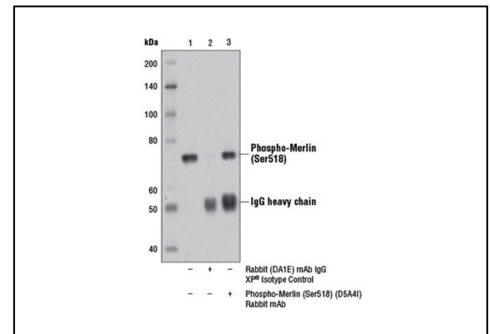
#13281

Phospho-Merlin (Ser518) (D5A4I) Rabbit mAb

Western blot analysis of extracts from PC-3 cells and wild-type (wt) and *Nf2* knockout (-/-) mouse embryonic fibroblasts (MEFs), untreated (-) or λ phosphatase-treated (+), using Phospho-Merlin (Ser518) (D5A4I) Rabbit mAb (upper), Merlin (D3S3W) Rabbit mAb #12888 (middle), or Ezrin/Radixin/Moesin Antibody #3142 (lower). (MEF wt and MEF *Nf2* (-/-) cells were kindly provided by Dr. Andrea McClatchey, MGH Cancer Center and Harvard Medical School, Charlestown MA).



Immunoprecipitation of Phospho-Merlin (Ser518) protein from PC-3 cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Phospho-Merlin (Ser518) (D5A4I) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Phospho-Merlin (Ser518) (D5A4I) Rabbit mAb.



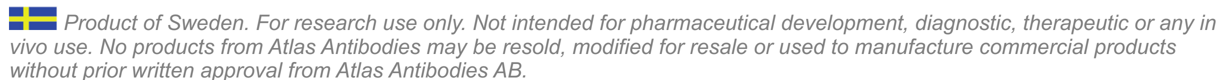
ATLAS ANTIBODIES

Anti-HDAC1 Product Datasheet

Polyclonal Antibody

PRODUCT SPECIFICATION

Product Name	Anti-HDAC1
Product Number	HPA029693
Gene Description	histone deacetylase 1
Clonality	Polyclonal
Isotype	IgG
Host	Rabbit
Antigen Sequence	Recombinant Protein Epitope Signature Tag (PrEST) antigen sequence: RIACEEEFSDSEEEGEGGRKNSSNFKKAKRVKTEDEKEKDPEEKKEVTEE EKTKEEKPEAKGVKE
Purification Method	Affinity purified using the PrEST antigen as affinity ligand
Verified Species Reactivity	Human
Recommended Applications	IHC (Immunohistochemistry) - Antibody dilution: 1:200 - 1:500 - Retrieval method: HIER pH6 WB (Western Blot) - Working concentration: 0.04-0.4 µg/ml ICC-IF (Immunofluorescence) - Fixation/Permeabilization: PFA/Triton X-100 - Working concentration: 0.25-2 µg/ml
Characterization Data	Available at atlasantibodies.com/products/HPA029693
Buffer	40% glycerol and PBS (pH 7.2). 0.02% sodium azide is added as preservative.
Concentration	Lot dependent
Storage	Store at +4°C for short term storage. Long time storage is recommended at -20°C.
Notes	Gently mix before use. Optimal concentrations and conditions for each application should be determined by the user. For protocols, additional product information, such as images and references, see atlasantibodies.com.



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SANTA CRUZ BIOTECHNOLOGY, INC.

Lamin B1 (B-10): sc-374015

SANTA CRUZ BIOTECHNOLOGY
The Power to Question

BACKGROUND

A unique family of cysteine proteases has been described that differs in sequence, structure and substrate specificity from any previously described protease family. This family, termed Ced-3/ICE, functions as key components of the apoptotic machinery and act to destroy specific target proteins which are critical to cellular longevity. Nuclear lamins are critical to maintaining the integrity of the nuclear envelope and cellular morphology as components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. B-type lamins undergo a series of modifications, such as farnesylation and phosphorylation. Increased phosphorylation of the lamins occurs before envelope disintegration and probably plays a role in regulating lamin associations. Nuclear Lamin B is fragmented as a consequence of apoptosis by an unidentified member of the ICE family.

CHROMOSOMAL LOCATION

Genetic locus: LMNB1 (human) mapping to 5q23.2; Lmnb1 (mouse) mapping to 18 D3.

SOURCE

Lamin B1 (B-10) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 559-586 at the C-terminus of Lamin B1 of mouse origin.

PRODUCT

Each vial contains 200 µg IgG_1 kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Lamin B1 (B-10) is available conjugated to agarose (sc-374015 AC), 500 µg/ 0.25 ml agarose in 1 ml, for IP; to HRP (sc-374015 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-374015 PE), fluorescein (sc-374015 FITC), Alexa Fluor® 488 (sc-374015 AF488), Alexa Fluor® 546 (sc-374015 AF546), Alexa Fluor® 594 (sc-374015 AF594) or Alexa Fluor® 647 (sc-374015 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-374015 AF680) or Alexa Fluor® 790 (sc-374015 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

Blocking peptide available for competition studies, sc-374015 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

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STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

APPLICATIONS

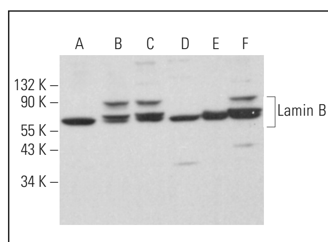
Lamin B1 (B-10) is recommended for detection of Lamin B1 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for Lamin B1 siRNA (h): sc-29386, Lamin B1 siRNA (m): sc-35779, Lamin B1 shRNA Plasmid (h): sc-29386-SH, Lamin B1 shRNA Plasmid (m): sc-35779-SH, Lamin B1 shRNA (h) Lentiviral Particles: sc-29386-V and Lamin B1 shRNA (m) Lentiviral Particles: sc-35779-V.

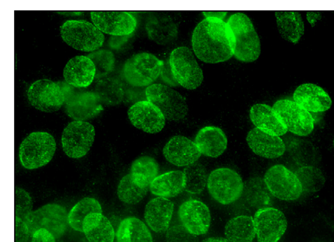
Molecular Weight of Lamin B1: 67 kDa.

Positive Controls: HL-60 whole cell lysate: sc-2209, C2C12 whole cell lysate: sc-364188 or F9 cell lysate: sc-2245.

DATA



Lamin B1 (B-10): sc-374015. Western blot analysis of Lamin B1 expression in HL-60 (**A**), C2C12 (**B**), F9 (**C**), CCRF-CEM (**D**), Ramos (**E**) and WR19L (**F**) whole cell lysates.



Lamin B1 (B-10): sc-374015. Immunofluorescence staining of methanol-fixed NIH/3T3 cells showing nuclear envelope localization.

SELECT PRODUCT CITATIONS

1. Evangelisti, C., et al. 2009. TIS21/BTG2/PC3 and cyclin D1 are key determinants of nuclear diacylglycerol kinase-ζ-dependent cell cycle arrest. Cell. Signal. 21: 801-809.
2. Jeong Nam, Y., et al. 2017. KATP channel block inhibits the Toll-like receptor 2-mediated stimulation of NFκB by suppressing the activation of Akt, mTOR, JNK and p38-MAPK. Eur. J. Pharmacol. 815: 190-201.
3. Zhang, Z., et al. 2018. PHACTR1 regulates oxidative stress and inflammation to coronary artery endothelial cells via interaction with NFκB/p65. Atherosclerosis 278: 180-189.
4. Tolkach, Y., et al. 2019. Apelin and apelin receptor expression in renal cell carcinoma. Br. J. Cancer 120: 633-639.
5. Mun, G.I., et al. 2020. Decreased expression of FBXW7 by ERK1/2 activation in drug-resistant cancer cells confers transcriptional activation of MDR1 by suppression of ubiquitin degradation of HSF1. Cell Death Dis. 11: 395.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

Technical Data Sheet

Purified Mouse Anti-β-Catenin

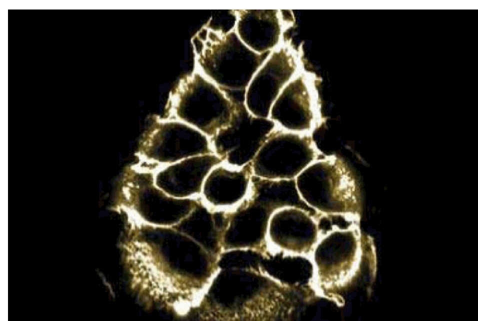
Product Information

Material Number:	**610153**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	14/Beta-Catenin
Immunogen:	Mouse β-Catenin aa. 571-781
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse, Rat, Dog, Chicken
Target MW:	92 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The 14/Beta-Catenin monoclonal antibody specifically binds to Beta-Catenin (β-Catenin). β-Catenin is a 92 kDa protein that binds to the cytoplasmic tail of E-Cadherin. The cadherins, transmembrane adhesion molecules, are found with catenins at adherens junctions (zonula adherens). Deletions in the cytoplasmic domain of E-Cadherin which eliminate catenin binding also result in a loss of cell adhesion, indicating that this binding is essential for E-Cadherin function. Although the α- and β-Catenins have been cloned, very little is known about their biochemical roles. However a link between β-Catenin and colon cancer has been described. β-Catenin was found to co-immunoprecipitate with the APC tumor suppressor protein in human colorectal tumor cell lines, as well as in human kidney 293 cells. E-Cadherin, however, was not detectable in these complexes. Thus the APC-Catenin complex may be affecting the transmission of contact inhibition signals and/or the regulation of cell adhesion.

Western blot analysis of β-Catenin on HeLa cell lysate. *Lane 1: 1:500, lane 2: 1:1000, lane 3: 1:2000 dilution of the Mouse Anti- β-Catenin antibody.*



Immunofluorescent staining of A431 cell line with the Anti- β-Catenin antibody.

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development
Immunofluorescence	Tested During Development
Immunohistochemistry	Tested During Development

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
611449	HeLa Cell Lysate	500 µg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Eger A, Stockinger A, Schaffhauser B, Beug H, Foisner R. Epithelial mesenchymal transition by c-Fos estrogen receptor activation involves nuclear translocation of beta-catenin and upregulation of beta-catenin/lymphoid enhancer binding factor-1 transcriptional activity. *J Cell Biol.* 2000; 148(1):173-187. (Clone-specific: Electron microscopy, Immunofluorescence, Immunoprecipitation, Western blot)

Fallone F, Britton S, Nieto L, Salles B, Muller C. ATR controls cellular adaptation to hypoxia through positive regulation of hypoxia-inducible factor 1 (HIF-1) expression. *Oncogene.* 2013; 32(37):4387-4396. (Clone-specific: Western blot)

Lee MS, D'Amour KA, Papkoff J. A yeast model system for functional analysis of beta-catenin signaling. *J Cell Biol.* 2002; 158(6):1067-1078. (Clone-specific: Immunofluorescence, Immunoprecipitation, Western blot)

Ozawa M, Ringwald M, Kemler R. Uvomorulin-catenin complex formation is regulated by a specific domain in the cytoplasmic region of the cell adhesion molecule. *Proc Natl Acad Sci U S A.* 1990; 87(11):4246-4250. (Biology)

Persad S, Troussard AA, McPhee TR, Mulholland DJ, Dedhar S. Tumor suppressor PTEN inhibits nuclear accumulation of beta-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation. *J Cell Biol.* 2001; 153(6):1161-1173. (Clone-specific: Gel shift, Immunofluorescence, Immunoprecipitation, Western blot)

Tateishi K, Omata M, Tanaka K, Chiba T. The NEDD8 system is essential for cell cycle progression and morphogenetic pathway in mice. *J Cell Biol.* 2001; 155(4):571-579. (Clone-specific: Immunofluorescence, Immunohistochemistry)

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RAD51 Monoclonal Antibody (14B4)

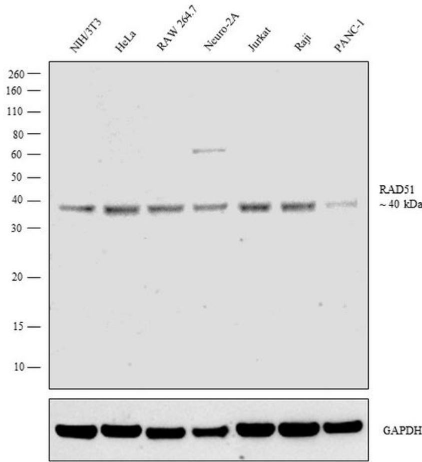
Product Details	
Size	100 µL
Species Reactivity	Chicken, Human, Mouse, Rat
Published Species	Human
Host/Isotype	Mouse / IgG2a
Class	Monoclonal
Type	Antibody
Clone	14B4
Conjugate	Unconjugated
Immunogen	Full length Rad51 protein (amino acids 1-338) expressed in E. coli.
Form	Liquid
Concentration	1.0 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS, pH 7
Contains	no preservative
Storage conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.
RRID	AB_560832

Applications	Tested Dilution	Publications
Western Blot (WB)	1:500-1:3,000	-
Immunohistochemistry (Paraffin) (IHC (P))	1:100-1:1,000	-
Immunocytochemistry (ICC/IF)	1:100-1:1,000	1 Publication
Immunoprecipitation (IP)	Assay-dependent	-
in situ PLA (PLA)	Assay-dependent	-

Product Specific Information

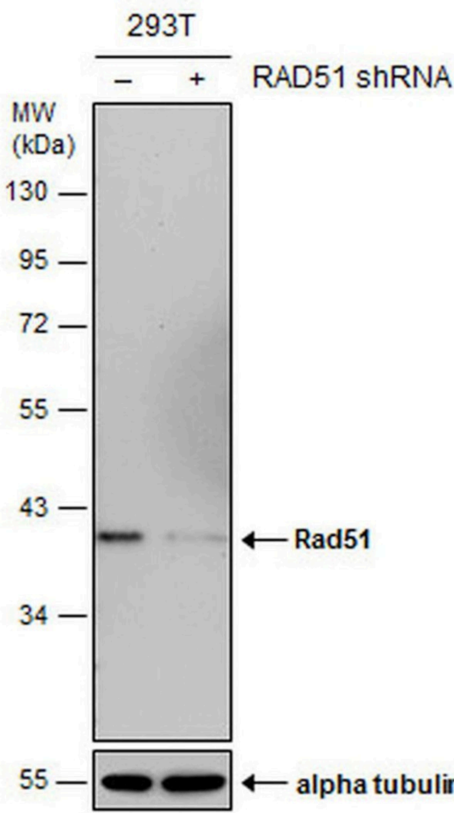
A suggested positive control for this product is T24.

Product Images For RAD51 Monoclonal Antibody (14B4)



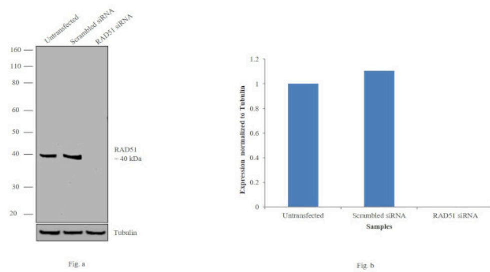
RAD51 Antibody (MA1-23271) in WB

Western blot analysis was performed on modified whole cell extracts (1% SDS) (30 µg lysate) of NIH/3T3 (Lane 1), HeLa (Lane 2), RAW 264.7 (Lane 3), Neuro-2A (Lane 4), Jurkat (Lane 5), Raji (Lane 6) and PANC-1 (Lane 7). The blot was probed with Anti-RAD51 Monoclonal Antibody (Product # MA1-23271, 1:1000 dilution) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg /mL, 1:4000 dilution). A 40 kDa band corresponding to RAD51 was observed in all the cell lines tested.



RAD51 Antibody (MA1-23271)

Antibody specificity was demonstrated by siRNA mediated knockdown of the target protein. 293T cells were transfected with RAD51 siRNA and decrease in signal intensity was observed in western blot application using RAD51 Antibody (Product # MA1-23271). {KD}



RAD51 Antibody (MA1-23271)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with RAD51 siRNA and reduction of signal was observed in Western Blot using RAD51 Monoclonal Antibody (14B4) (Product # MA1-23271). {KD}

View more figures on thermofisher.com

1 Reference

Immunocytochemistry (1)

Genes & development

A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage.

"MA1-23271 was used in immunocytochemistry to study the response to double-stranded breaks at the nucleolar organizer regions."

Authors: van Sluis M,McStay B

Year
2015

Species
Human

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Technical Data Sheet

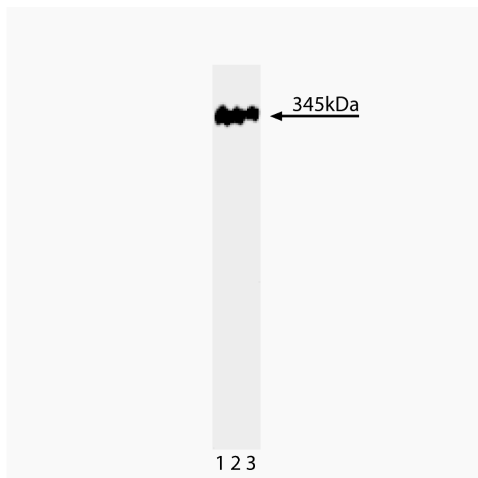
Purified Mouse Anti-Human 53BP1

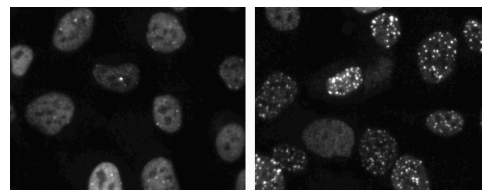
Product Information

Material Number:	**612522**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	19/53BP1
Immunogen:	Human 53BP1 aa. 149-259
Isotype:	Mouse IgG2b
Reactivity:	QC Testing: Human
Target MW:	345 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The p53 protein is critical to regulation of normal cell growth and is a suppressor of tumor cell proliferation. Inactivation of p53 by a number of mechanisms, such as missense mutations or interaction with oncogenic viral or cellular proteins, can result in tumor progression. In addition, Bcl2 and p53 are involved in apoptosis in an antagonistic fashion such that overexpressed Bcl2 inhibits p53-induced apoptosis. 53BP1 and 53BP2 were identified in a yeast two-hybrid screen of proteins that bind p53. Both 53BP1 and 53BP2 bind wild type p53, but not mutant p53 found in tumor cells. p53BP1 is localized to the cytoplasm and nucleus, while p53BP2 is found only in the cytoplasm. 53BP1 has BRCT motifs found in proteins involved in cell cycle control and DNA repair. DNA damage leads to 53BP1 hyperphosphorylation, which may be mediated by ATM. 53BP2 has four ankyrin repeats and a SH3 domain that are required for interactions with Bcl2 and p53. Overexpression of 53BP2 in 293 cells inhibits progression of the cell cycle in G2/M phase, while co-transfection of 53BP2 with p53 in H358 cells enhances p53-mediated transcriptional activation. The interaction between 53BP2 and p53 may be regulated by Bcl2, since competition experiments demonstrate that Bcl2 prevents p53 binding to 53BP2. In addition, 53BP2 can also bind the apoptotic-related p65 subunit of NFκB and this subunit can inhibit 53BP2-induced cell death.





Left Figure: Western blot analysis of 53BP1 on a HeLa lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the anti-53BP1 antibody.* ***Right Figure: Immunofluorescent staining of HT1080 cells (ATCC CCL-121).*** *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were either mock treated (PBS, left) or exposed to hydrogen peroxide (400uM, right) for 30 minutes and allowed to recover in media for 30 minutes. After treatment cells were stained using the alcohol perm protocol and the anti-53BP1 antibody. The second step reagent was Alexa Fluor® 488 goat anti-mouse IgG (Invitrogen). The image is a confocal collapsed stack, taken on a BD Pathway™ 855 bioimaging system with a 40x objective. This antibody also stains A549 (ATCC CCL-158), HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
611449	HeLa Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Triton is a trademark of the Dow Chemical Company.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Iwabuchi K, Bartel PL, Li B, Marraccino R, Fields S. Two cellular proteins that bind to wild-type but not mutant p53. *Proc Natl Acad Sci U S A.* 1994; 91(13):6098-6102. (Biology)

Iwabuchi K, Li B, Massa HF, Trask BJ, Date T, Fields S. Stimulation of p53-mediated transcriptional activation by the p53-binding proteins, 53BP1 and 53BP2. *J Biol Chem.* 1998; 273(40):26061-26068. (Biology)

Rappold I, Iwabuchi K, Date T, Chen J. Tumor suppressor p53 binding protein 1 (53BP1) is involved in DNA damage-signaling pathways. *J Cell Biol.* 2001; 153(3):613-620. (Biology)

Ki-67 Recombinant Rabbit Monoclonal Antibody (SP6)

Product Details	
Size	500 µL
Species Reactivity	Human, Mouse, Rat
Published Species	Rat, Pig, Non-human primate, Hamster, Bovine, Sheep, Cat, Mouse, Human, Rhesus monkey, Guinea pig, Dog, Rabbit
Host/Isotype	Rabbit / IgG
Expression system	proprietary
Class	Recombinant Monoclonal
Type	Antibody
Clone	SP6
Conjugate	Unconjugated
Immunogen	Synthetic peptide within Human Ki67 aa 1200-1300
Form	Liquid
Concentration	0.031 mg/mL
Purification	Protein A
Storage buffer	PBS, pH 7.2, with 1% BSA
Contains	0.1% sodium azide
Storage conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.
RRID	AB_10979488

Applications	Tested Dilution	Publications
Western Blot (WB)	1:100	12 Publications
Immunohistochemistry (IHC)	-	634 Publications
Immunohistochemistry (Paraffin) (IHC (P))	1:100-1:200	53 Publications
Immunohistochemistry (Frozen) (IHC (F))	-	11 Publications
Immunocytochemistry (ICC/IF)	1:250-1:500	69 Publications
Flow Cytometry (Flow)	1:1,000	7 Publications
Neutralization (Neu)	-	1 Publication
Functional Assay (FN)	-	1 Publication
Miscellaneous PubMed (Misc)	-	3 Publications

Product Specific Information

Staining of formalin-fixed tissues requires boiling tissue section in 10 mM citrate buffer, pH 6.0 for 10-20 minutes followed by cooling at room temperature for 20 minutes.

Recommended positive controls:

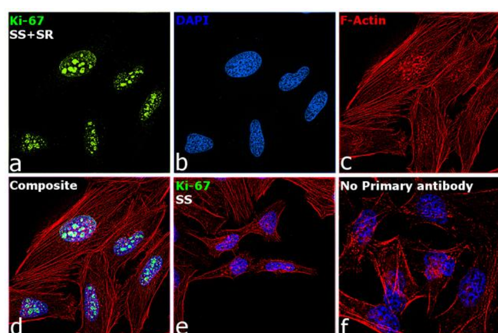
IHC (P) - Human tonsil and testis tissue, common marmoset spleen tissue, rat esophagus, small intestine and liver tissue, mouse embryonic skin tissue

IHC (F) - Rat lymph node tissue, transgenic mouse spinal cord tissue

ICC/IF - HAP1 cells, human cardiac stem cells, HEp-2 cells, rat cardiomyocytes

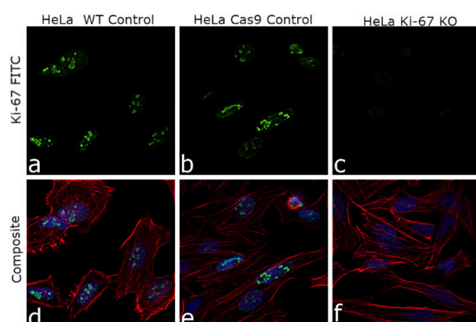
Flow - HAP1 cells

Product Images For Ki-67 Recombinant Rabbit Monoclonal Antibody (SP6)



Ki-67 Antibody (MA5-14520)
Detection of altered expression of target protein by cell treatment demonstrates antibody specificity. Immunofluorescence analysis of Ki-67 using Ki-67 Monoclonal Antibody (SP6) (Product # MA5-14520) shows increased expression of Ki-67 in HeLa cell line upon serum starvation (36 hours) followed by serum release (6 hours). By comparison, reduced expression of Ki-67 was seen in HeLa cell line upon serum starvation (36 hours) alone. {TM}

Ki-67 Antibody (MA5-14520) in IHC (P)
Immunohistochemical analysis of Ki-67 was performed using formalin-fixed paraffin-embedded human colon adenocarcinoma tissue sections. To expose the target protein, heat-induced epitope retrieval was performed on de-paraffinized sections using eBioscience™ IHC Antigen Retrieval Solution - High pH (10X) (Product # 00-4956-58) diluted to 1X solution in water in a decloaking chamber at 110 degree Celsius for 15 minutes. Following antigen retrieval, the sections were blocked with 2% normal goat serum in 1X PBS for 45 minutes at room temperature and then probed with or without Ki-67 Recombinant Rabbit Monoclonal Antibody (SP6) (Product # MA5-14520) at 1:100 dilution in 0.1% normal goat serum overnight at 4 degree Celsius in a humidified chamber. Detection was performed using Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32731) at a dilution of 1:2000 in 0.1% normal goat serum for 45 minutes at room temperature. ReadyProbes™ Tissue Autofluorescence Quenching Kit (Product # R37630) was used to quench autofluorescence from the tissues. Nuclei were stained with DAPI (Product # D1306) and the sections were mounted using ProLong™ Glass Antifade Mountant (Product # P36984). The images were captured on EVOS™ M7000 Imaging System (Product # AMF7000) at 20X magnification and externally deconvoluted.



Ki-67 Antibody (MA5-14520)
Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in Ki-67 KO cell line compared to control cell line using Ki-67 Recombinant Rabbit Monoclonal Antibody (SP6) (Product # MA5-14520). {KO}

View more figures on thermofisher.com

791 References

Western Blot (12)

Journal of immunology research

Knockdown of lncRNA CCAT1 Inhibits the Progression of Colorectal Cancer via hsa-miR-4679 Mediating the Downregulation of GNG10.

"MA5-14520 was used in Western Blot, Immunohistochemistry to reveal that lncRNA CCAT1 facilitated colorectal cancer progression via the hsa-miR-4679/GNG10 axis and provided new potential therapeutic targets for colorectal cancer."

Authors: Wang N,Li J,He J,Jing YG,Zhao WD,Yu WJ,Wang J

Year 2023
Species Human
Dilution 1:1000

OncoTargets and therapy

Acetone Extract of *Cornus officinalis* Leaves Exerts Anti-Melanoma Effects via Inhibiting STAT3 Signaling.

"MA5-14520 was used in Western Blot to investigate the intervention and mechanism of 50% acetone extract of C. officinalis leaves (SZYY) on melanoma xenografts."

Authors: Xu R,Zeng M,Wu Y,Wang S,Zhang B,Zhang J,Kan Y,Li B,Cao B,Zheng X,Feng W

Year 2022
Species Mouse
Dilution 1:100

View more WB references on thermofisher.com

Immunohistochemistry (634)

Nature aging

Transcriptional and epigenetic decoding of the microglial aging process.

"MA5-14520 was used in Immunohistochemistry to map the transcriptional and epigenetic profiles of microglia from 3- to 24-month-old mice."

Authors: Li X,Li Y,Jin Y,Zhang Y,Wu J,Xu Z,Huang Y,Cai L,Gao S,Liu T,Zeng F,Wang Y,Wang W,Yuan TF,Tian H,Shu Y,Guo F,Lu W,Mao Y,Mei X,Rao Y,Peng B

Year 2023
Species Mouse
Dilution 1:250

Nature communications

Astroglial Hmgb1 regulates postnatal astrocyte morphogenesis and cerebrovascular maturation.

"MA5-14520 was used in Immunohistochemistry-immunofluorescence to identify astroglial Hmgb1 as an important player in postnatal gliovascular maturation."

Authors: Freitas-Andrade M,Comin CH,Van Dyken P,Ouellette J,Raman-Nair J,Blakeley N,Liu QY,Leclerc S,Pan Y,Liu Z,Carrier M,Thakur K,Savard A,Rurak GM,Tremblay MÈ,Salmaso N,da F Costa L,Coppola G,Lacoste B

Year 2023
Species Mouse
Dilution 1:250

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More applications with references on thermofisher.com

IHC (P) (53) | IHC (F) (11) | ICC/IF (69) | Flow (7) | Neu (1) | FN (1) | Misc (3)

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Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
WB, IP, IHC-P, IF-IC, FC-FP	H	Endogenous	55	Rabbit IgG	#P20248	890

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:800 - 1:3200
Immunofluorescence (Immunocytochemistry)	1:400 - 1:1600
Flow Cytometry (Fixed/Permeabilized)	1:400 - 1:1600

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

For a carrier free (BSA and azide free) version of this product see product #29113.

Specificity / Sensitivity

Cyclin A2 (E6D1J) XP® Rabbit mAb recognizes endogenous levels of total cyclin A2 protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with recombinant protein specific to the amino terminus of human cyclin A2 protein. The epitope corresponds to a region surrounding Glu121 of human cyclin A2.

Background

While overcoming the G1/S checkpoint to commence DNA replication requires cyclin E, and traversing the G2/M checkpoint to initiate mitosis requires cyclin B to be present, cyclin A seems to be required for both S-phase and M-phase (1). A number of studies have described the ability of overexpressed cyclin A to accelerate the G1 to S transition, causing DNA replication, and cyclin A antisense DNA can prevent DNA replication (2-4). Cyclin A availability is apparently the rate-limiting step for entry into mitosis, and cyclin A is required for the completion of prophase (5). At late prophase, cyclin A may no longer be necessary as cdc2/cyclinB1 becomes active (5).

Background References

1. Pagano, M. et al. (1992) *EMBO. J.* 11, 961-71.
2. Resnitzky, D. et al. (1995) *Mol. Cell. Biol.* 15, 4347-52.
3. d'Urso, G. et al. (1990) *Science* 250, 786-91.
4. Zindy, F. et al. (1992) *Biochem. Biophys. Res. Commun.* 182, 1144-54.
5. Furuno, N. et al. (1999) *J. Cell. Biol.* 147, 295-306.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

WB: Western Blotting **IP:** Immunoprecipitation **IHC-P:** Immunohistochemistry (Paraffin)
IF-IC: Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken
Dm: D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae
Ce: C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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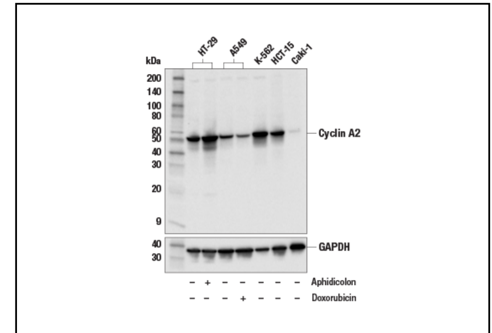
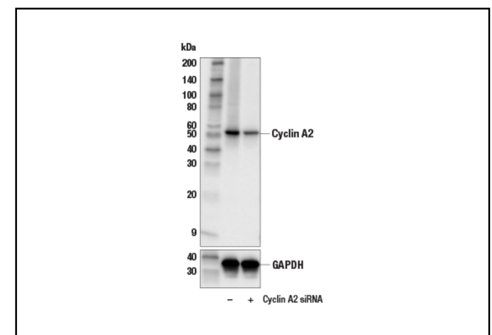
Cyclin A2 (E6D1J) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

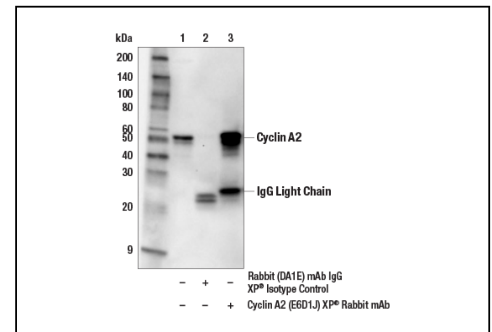
Western blot analysis of extracts from various human cell lines, untreated (-) or treated with Aphidicolin #32774 (10 µg/mL, 24 hr; +) or Doxorubicin #5927 (0.5 µM, 24 hr; +), using Cyclin A2 (E6D1J) XP® Rabbit mAb (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). Cyclin A2 protein is induced with aphidicolin and reduced with doxorubicin as expected. Low expression of cyclin A2 protein in Caki-1 cells is consistent with the predicted expression pattern.



Western blot analysis of extracts from HCT 116 cells, transfected with control siRNA (-) or cyclin A2 siRNA (+), using Cyclin A2 (E6D1J) XP® Rabbit mAb (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower).



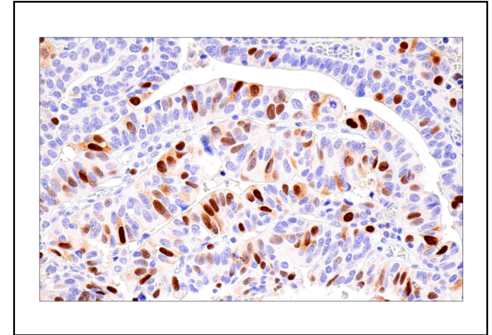
Immunoprecipitation of cyclin A2 protein from HCT 116 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is Cyclin A2 (E6D1J) XP® Rabbit mAb. Western blot analysis was performed using Cyclin A2 (E6D1J) XP® Rabbit mAb. Mouse Anti-Rabbit IgG (Light-Chain Specific) (D4W3E) mAb (HRP Conjugate) #93702 was used as the secondary antibody.



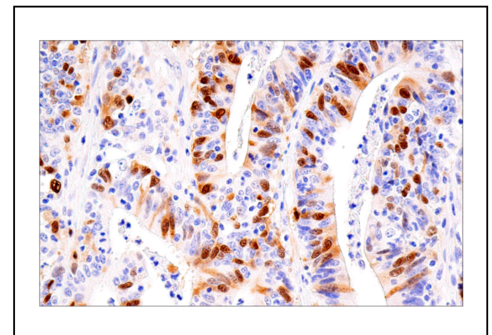
#67955

Cyclin A2 (E6D1J) XP® Rabbit mAb

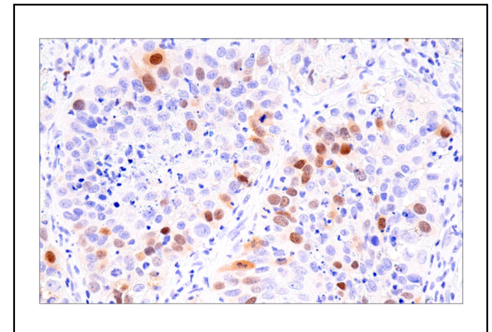
Immunohistochemical analysis of paraffin-embedded human urothelial carcinoma using Cyclin A2 (E6D1J) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Cyclin A2 (E6D1J) XP® Rabbit mAb.



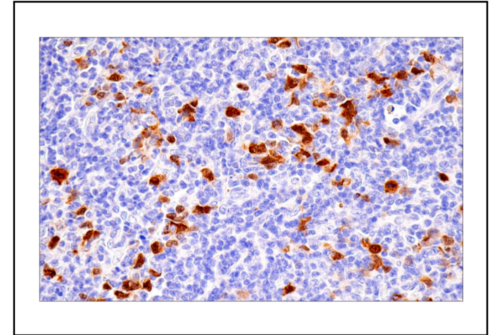
Immunohistochemical analysis of paraffin-embedded human non-small cell lung carcinoma using Cyclin A2 (E6D1J) XP® Rabbit mAb.



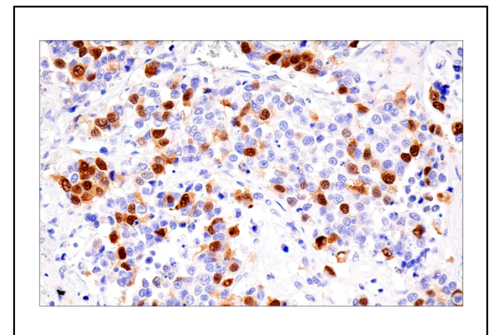
#67955

Cyclin A2 (E6D1J) XP® Rabbit mAb

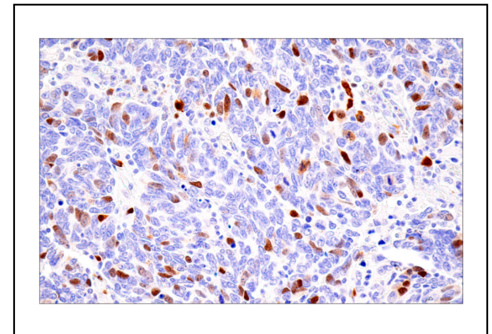
Immunohistochemical analysis of paraffin-embedded human non-Hodgkin lymphoma using Cyclin A2 (E6D1J) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human large cell neuroendocrine carcinoma using Cyclin A2 (E6D1J) XP® Rabbit mAb.



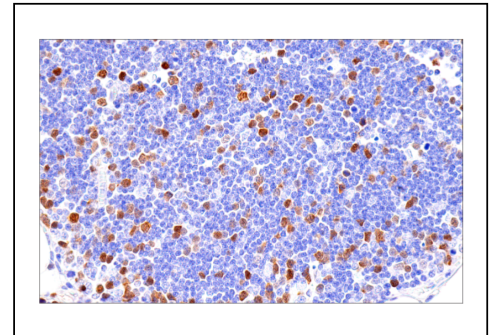
Immunohistochemical analysis of paraffin-embedded human small cell carcinoma of the salivary gland using Cyclin A2 (E6D1J) XP® Rabbit mAb.



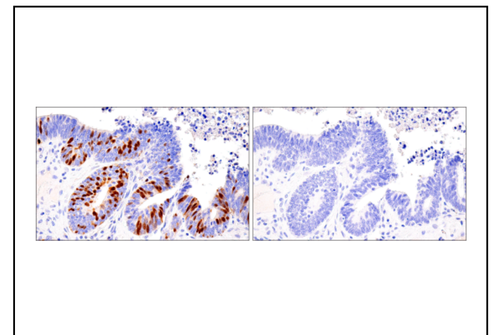
Revision 8

#67955

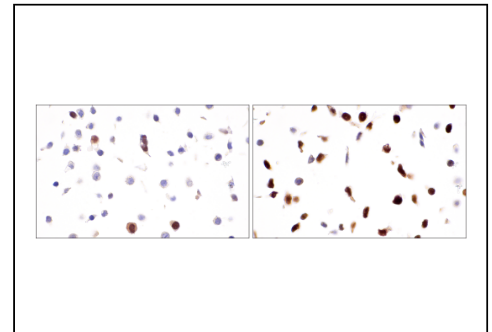
Cyclin A2 (E6D1J) XP® Rabbit mAb



Immunohistochemical analysis of paraffin-embedded normal human thymus using Cyclin A2 (E6D1J) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human endometrioid adenocarcinoma using Cyclin A2 (E6D1J) XP® Rabbit mAb (left) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (right).



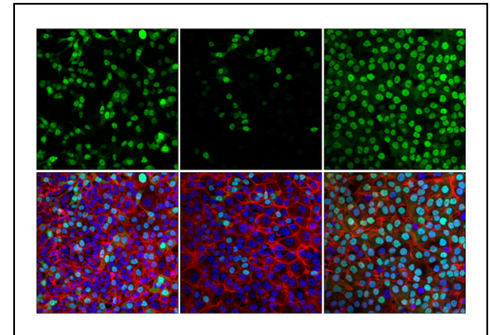
Immunohistochemical analysis of paraffin-embedded HT-29 cell pellets, untreated (left) or treated with Aphidicolin #32774 (10 µg/ml, 24 hr; right), using Cyclin A2 (E6D1J) XP® Rabbit mAb.

Revision 8

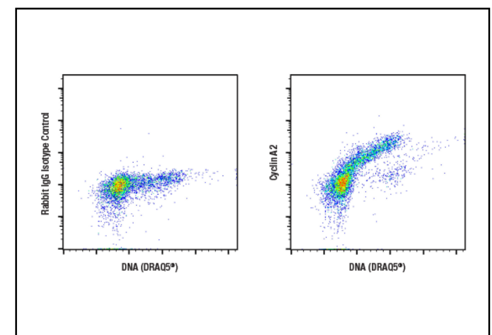
#67955

Cyclin A2 (E6D1J) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®



Confocal immunofluorescent analysis of HCT 116 cells, either mock transfected (left, moderate-expressing), transfected with siRNA directed against human cyclin A2 (center, low-expressing), or treated with Aphidicolin #32774 (10 µg/mL, 24 hr; right, high-expressing), using Cyclin A2 (E6D1J) XP® Rabbit mAb (green), DyLight™ 650 Phalloidin #12956 (red), and DAPI #4083 (blue).



Flow cytometric analysis of Jurkat cells using DRAQ5® #4084 and Cyclin A2 (E6D1J) XP® Rabbit mAb (right) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (left). Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor 488® Conjugate) #4412 was used as a secondary antibody.

Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488

Product Details	
Size	1 mg
Species Reactivity	Mouse
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 488
Excitation/Emission Max	499/520 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_2534069

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	Assay-dependent	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	-	0 Publication
Immunohistochemistry - Free Floating (IHC (Free))	-	0 Publication
Immunocytochemistry (ICC/IF)	1 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
in situ PLA (PLA)	-	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

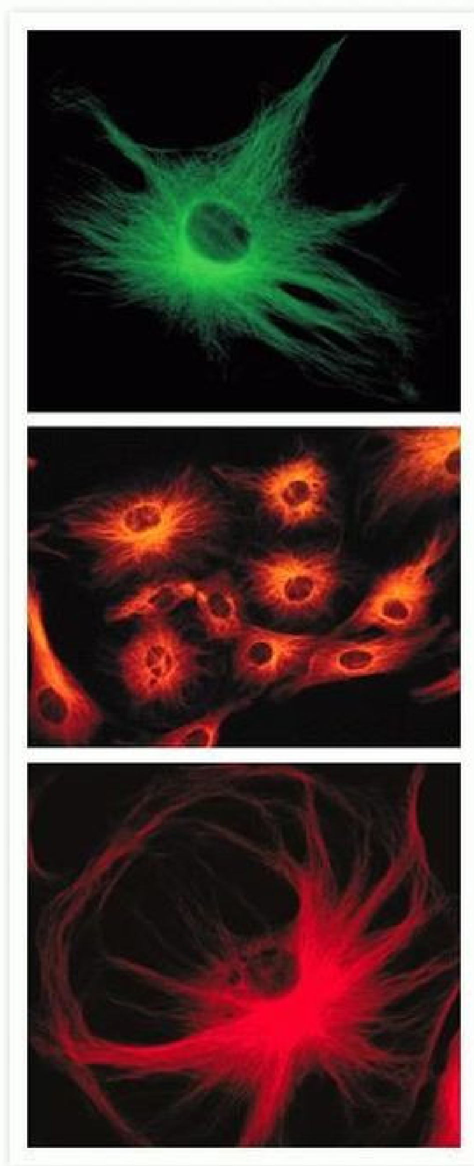
To minimize cross-reactivity, these goat anti-mouse IgG whole antibodies have been cross-adsorbed against human IgG and human serum. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there are may be the presence of endogenous immunoglobulins. For a highly cross-adsorbed secondary antibody equivalent, please see product Cat. No. A11029.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 488 dye is a bright, green-fluorescent dye with excitation ideally suited to the 488 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 488 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 488 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.

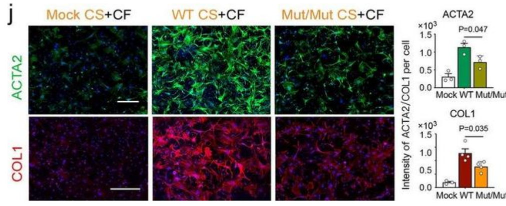
Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

Product will be shipped at Room Temperature.

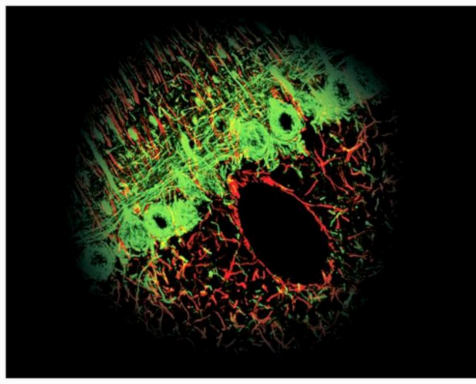
Product Images For Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in ICC/IF
Microtubules of bovine pulmonary artery endothelial cells tagged with mouse monoclonal anti-a-tubulin antibody (Product # A11126) and subsequently probed with: Alexa Fluor® 488 Goat Anti-Mouse IgG antibody (Product # A-11001, top panel), Alexa Fluor® 546 Goat Anti-Mouse IgG antibody (Product # A-11003, middle panel) or Alexa Fluor® 594 Goat Anti-Mouse IgG antibody (Product # A-11005, bottom panel). These images were acquired using a FITC bandpass optical filter set, a rhodamine bandpass optical filter set, and a Texas Red bandpass optical filter set, respectively.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in ICC/IF
WWP2 regulates macrophage activation and profibrotic function.a Scatter plot of log2fold changes (FC) in mRNA expression from scRNA-seq in cardiac macrophages between Ang-II-treated Mut/Mut and WT mice (y axis) and log2FC between WT Ang-II-treated and WT untreated mice (x axis). Differentially expressed genes (DEGs) in red (n = 237, FDR < 0.05). Ang-II treatment: 500 ng /kg/min, 7 days. b Top downregulated pathways in Mut/Mut macrophages identified by gene set enrichment analysis (GSEA) of DEGs. NES, normalized enrichment score. c Violin plots illustrate the expression score of the GSEA-derived pathways across all cardiac macrophage clusters in Mut/Mut and WT mice after treatment with Ang-II (7 days). d qRT-PCR analysis of selected pro-inflammatory and homeostatic/reparatory genes in macrophages sorted from LV of WT and Mut/Mut mice treated with saline or Ang-II (7 days). n = 5-12 for each group. e Representative immunofluorescence staining of smooth muscle aortic alpha-actin (ACTA2, green) in (myo)fibroblasts co-cultured with CD45 + macrophages (red). Scale bar, 100 µm. f Number of cardiac macrophages moving across the proximity border (left), and ACTA2 expression in (myo) fibroblasts (right). n = 3 per experimental group and 15-25 fibroblast images were taken from each slide. g Schematic of the co-culture experimental setup in vitro. The conditioned supernatant (CS) from BMDMs treated with LPS (100 ng/ml, 4 hrs) and I… Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36450710), licensed under a CC BY license.



Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11001) in ICC/IF
Filamentous structures of neuronal cells in a rat cerebellum were fluorescently labeled to differentiate the cell types. The cerebellum section was probed with primary antibodies to neurofilament and glial fibrillary acidic proteins (GFAP) and subsequently visualized with the green-fluorescent Alexa Fluor® 488 Goat Anti-Mouse IgG (Product # A-11001) and red-orange-fluorescent Alexa Fluor® 568 Goat Anti-Rabbit IgG (Product # A-11011) antibodies. This confocal micrograph was contributed by Gillian Davidson, Andrew Hubbard and Chris Guerin, Neurotoxicology Group, M.R.C Toxicology Unit, University of Leicester, Leicester, U.K.

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7866 References

Generation of induced pluripotent stem cells from an individual with early onset and severe hypertrophic cardiomyopathy linked to MYBPC3: c.772G>A mutation. Hum Cell (2024)

Ganoderma lucidum extract attenuates corticotropin-releasing hormone-induced cellular senescence in human hair follicle cells. iScience (2024)

Effect of RNF113A deficiency on oxidative stress-induced NRF2 pathway. Anim Cells Syst (Seoul) (2024)

A human neural crest model reveals the developmental impact of neuroblastoma-associated chromosomal aberrations. Nat Commun (2024)

Sigma-1 Receptor Inhibition Reduces Mechanical Allodynia and Modulate Neuroinflammation in Chronic Neuropathic Pain. Mol Neurobiol (2024)

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Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 568

Product Details	
Size	1 mg
Species Reactivity	Rabbit
Host/Isotype	Goat / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Alexa Fluor™ 568
Excitation/Emission Max	579/603 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	liquid
Concentration	2 mg/mL
Purification	purified
Storage buffer	PBS, pH 7.5
Contains	5mM sodium azide
Storage conditions	4° C, store in dark
RRID	AB_143157

Applications	Tested Dilution	Publications
Western Blot (WB)	-	0 Publication
Immunohistochemistry (IHC)	-	0 Publication
Immunohistochemistry (Paraffin) (IHC (P))	-	0 Publication
Immunohistochemistry (PFA fixed) (IHC (PFA))	-	0 Publication
Immunohistochemistry (Frozen) (IHC (F))	Assay-dependent	0 Publication
Immunocytochemistry (ICC/IF)	2 µg/mL	0 Publication
Flow Cytometry (Flow)	1-10 µg/mL	0 Publication
Miscellaneous PubMed (Misc)	-	0 Publication

Product Specific Information

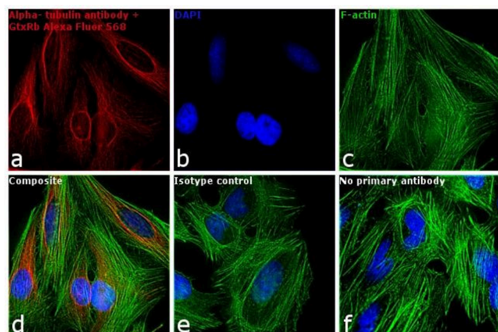
To minimize cross-reactivity, these goat anti-rabbit IgG whole antibodies have been cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there are may be the presence of endogenous immunoglobulins.

Alexa Fluor dyes are among the most trusted fluorescent dyes available today. Invitrogen™ Alexa Fluor 568 dye is a bright, orange/red-fluorescent dye with excitation ideally suited to the 568 nm laser line. For stable signal generation in imaging and flow cytometry, Alexa Fluor 568 dye is pH-insensitive over a wide molar range. Probes with high fluorescence quantum yield

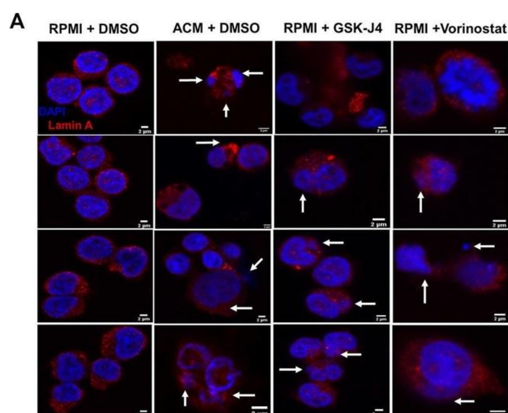
and high photostability allow detection of low-abundance biological structures with great sensitivity. Alexa Fluor 568 dye molecules can be attached to proteins at high molar ratios without significant self-quenching, enabling brighter conjugates and more sensitive detection. The degree of labeling for each conjugate is typically 2-8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the certificate of analysis for each product lot.

Using conjugate solutions: Centrifuge the protein conjugate solution briefly in a microcentrifuge before use; add only the supernatant to the experiment. This step will help eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining. Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore-labeled antibodies a final concentration of 1-10 µg/mL should be satisfactory for most immunohistochemistry and flow cytometry applications.

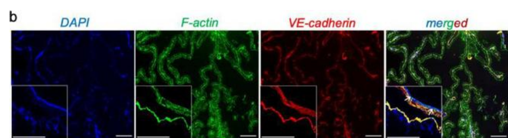
Product will be shipped at Room Temperature.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11011) in ICC/IF
Immunofluorescence analysis of Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor® 568 conjugate was performed using HeLa cells stained with alpha Tubulin Rabbit Polyclonal Antibody (Product # PA516891) The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 1% BSA for 1 hour and labeled with 2 µg/mL primary antibody for 3 hours at room temperature. Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor® 568 conjugate (Product # A-11011) was used at a concentration of 2 µg/mL in phosphate buffered saline containing 0.2% BSA for 45 minutes at room temperature, for detection of alpha Tubulin in the cytoplasm (Panel a: red). Nuclei (Panel b: blue) were stained with DAPI in SlowFade® Gold Antifade Mountant (Product # S36938). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379), 1:300) (Panel c: green). Panel d represents the composite image. No nonspecific staining was observed with the secondary antibody alone (panel f), or with an isotype control (panel e). The images were captured at 60X magnification.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11011) in ICC/IF
Treating human T-ALL cells with GSK-J4 and vorinostat phenocopy ACM-induced DNA damage and cytotoxicity in leukemia cells. Jurkat T cells were cultured in RPMI + DMSO, ACM + DMSO, or with RPMI + epigenetic modifying drugs (GSK-J4 or Vorinostat) for 48 h. The cells were then stained with lamin A with DAPI to visualize nuclei. (A) Representative images are shown with white arrows indicating nuclei spillage or fragmented nuclei. The percentage of cells harboring fragment nuclei, calculated by dividing the # of cells containing fragmented nuclei/total # of cell counted, is shown in (B). (C,D) Human T-ALL cells (Jurkat, Loucy, and Peer) were treated with DMSO (control), GSK-J4 (a histone demethylase inhibitor), or vorinostat (a histone deacetylase inhibitor) for 72 h. The percentage of dead cells after 3 days of treatment was determined using Annexin-V/PI staining flow by flow cytometry. Representative primary data from one of three independent experiments are shown in (C) with quantitative data from combined experiments presented in (D). Statistical significance was calculated using a one-way ANOVA followed by Tukey's multiple comparison post-test. **p < 0.01 and ****p < 0.0001. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/36060800), licensed under a CC BY license.



Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (A-11011) in ICC/IF
The biocompatibility of the BETA scaffold. (a) Scanning electron microscopy (SEM) of the BETA scaffold, which has already been introduced by us elsewhere [21]. The cells were populated onto and into the porous BETA scaffold with alveolar epithelial type II-like A549 cells within 5 days. The scale bars of the SEM images are 50 µm. (b) The chick chorioallantoic membrane (CAM) assay was used to evaluate the biocompatibility of the BETA scaffold. The immunofluorescence (IF) analysis showed the formation of blood vessels on the BETA scaffold characterized by VE-cadherin (red). Cell nuclei are shown in blue (DAPI) and F-actin cytoskeleton in green. (c,d) The CAM-deposited ECM and connective tissue on the membrane analyzed by Masson's trichrome analysis. (c, d) show the cross-sectioned and diagonally cross-sectioned view of the CAM and BETA scaffold, respectively. Cell nuclei are shown in dark blue/dark brown and connective tissue in green. The scale bars in (b-d) are 100 µm. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35892691), licensed under a CC BY license.

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2778 References

Lowering Hippocampal miR-29a Expression Slows Cognitive Decline and Reduces Beta-Amyloid Deposition in 5×FAD Mice. Mol Neurobiol (2024)

Implantation of Adipose-Derived Mesenchymal Stromal Cells (ADSCs)-Lining Prosthetic Graft Promotes Vascular Regeneration in Monkeys and Pigs. Tissue Eng Regen Med (2024)

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Bio-Rad Laboratories, Inc.

Certificate of Analysis

Material Description:	Goat Anti-Mouse IgG-HRP Conjug, BG, 2ml
Material Number:	1706516
Batch Number:	64545005
Manufacture Date:	2023-03-29
Expiration Date:	2026-03-28

Characteristic	**Results**
Binding activity at 490nm 0.12U/min	Pass
Blotting immunoassay, mouse IgG 3.9ng	Pass

Gloria V Cruz

This certificate has been verified and electronically approved by an authorized Quality representative. This e-signature is performed within a secure system.

2023-04-05 / 14:43:17 UTC

Date/Time

Bio-Rad Laboratories, Inc.

Certificate of Analysis

Material Description:	Goat Anti-Rabbit IgG-HRP Conjug, BG, 2ml
Material Number:	1706515
Batch Number:	64582898
Manufacture Date:	2023-11-10
Expiration Date:	2026-11-09

Characteristic	**Results**
Binding activity at 490nm 0.12U/min	Pass

Andrew Concepcion

This certificate has been verified and electronically approved by an authorized Quality representative. This e-signature is performed within a secure system.

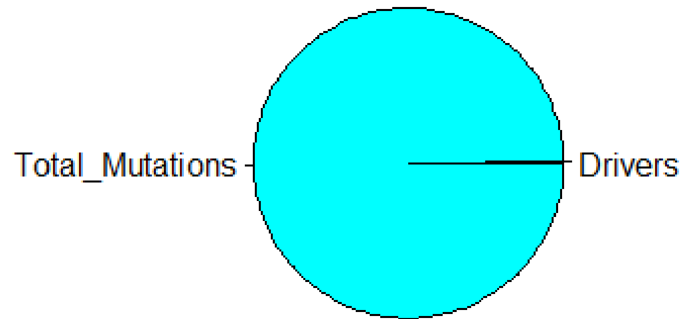
2023-11-27 / 18:33:19 UTC

Date/Time

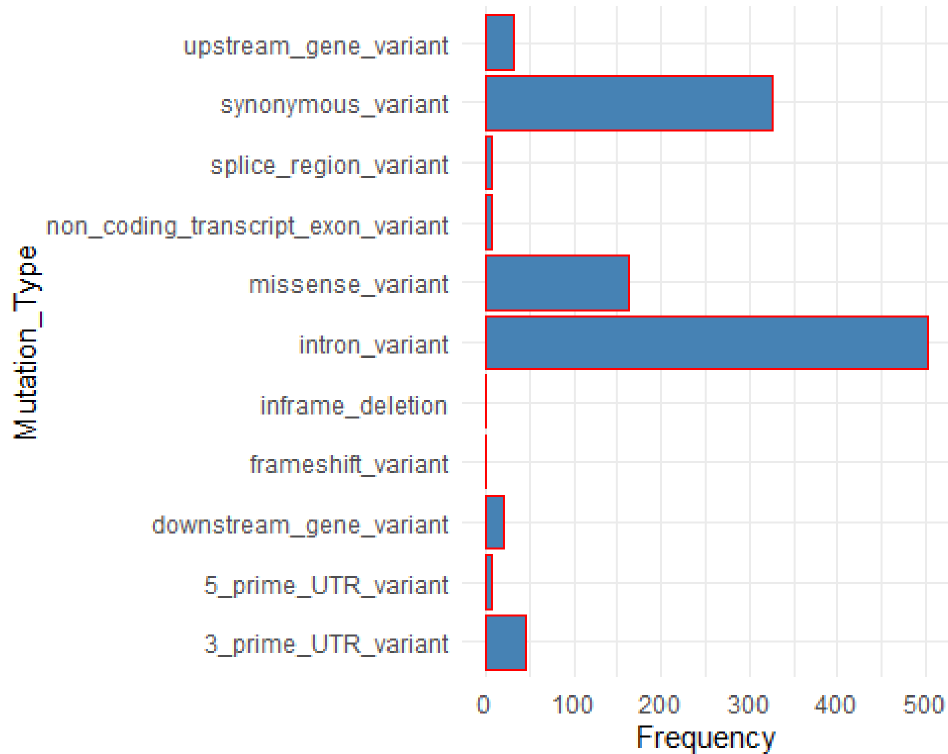
BTB0058 cell from patient with meningioma Sample B2135659

Maryam Shah

2023-06-27



Tumor_Sample_Barcode	Patient_ID	Pub_ID	Age	Gender	Tumour_Location	Histopathological_Subtype	Histopathological_grade	western_results
B2135659	BTB0058	MN496	57	Male	LEFT temporal convexity meningioma	unknown	I	NA



: High Frequency pathogenic Mutations

Chr	Start	End	Ref	Alt	Gene.refGene	ExonicFunc.refGene	AAChange.refGene	dbSNP	COSMIC_ID	AF
chr22	29091857	29091857	G	-	CHEK2	frameshift deletion	CHEK2:NM_001349956:exon10:c.899delC:p.T300Mfs*15	rs555607708	COSM5967258	0.56164

Notes

- some NF2 mutations have not been identified in Cancer Genome Interpreter (CGI) as driver mutations [1]. Gene with (p) means that mutation has been identified as "Passenger"
- The NF2 gene has been analyzed differently and all passenger or non-protein affecting mutations are reported due to the majority of NF2 mutations remain classified as variants of uncertain significance in clinical databases[2]. Please check all the reported NF2 mutations in this table for causing non-functioning Merlin protein by Western blotting validation i.e no visible phosphoNF2 or total NF2 bands.

Final Analysis tables are available on the Oliver SharePoint drive - Biomedical Research Laboratories - Oliver Hanemann\CLAIRE ADAMS\Bristol NGS 2020\TSU500_analysis\Maryam file summary tables for variants

Definitions

1. Type of mutation abbreviations; SNV; Single nucleotide variant; MNV, multiple nucleotide variant; INS; Insertion

2. dbSNP- submitted and annotated by dbSNP are given rs ID.
3. COSMIC ID- ID given by COSMIC (Catalogue of Somatic Mutations in Cancer) database
4. FATHMM_MKL score - A score that indicates the functional consequence of the mutation. The score ranges from 0 to 1. Mutations with score 0.5 are classified as neutral and above 0.5 are consider either deleterious or pathogenic. The most significant pathogenic mutations score are 0.7 [3-6]
5. Variant Allele freq (VAF)- Frequency of altered base in VCF (variant calling format) file in each tumor DNA. Due to the high error rate of NGS at the per-base call level, calls supported by less than 5% variant reads are typically considered to be likely false positive calls [7].
6. MAF (Minor Allele Frequency) - Frequency of the allele in the general population from either the Exome Aggregation Consortium (ExAC) or dbSNP (which uses 1,000 genome project allele frequencies). databases. For this database we have used a generous cutoff of 5% (0.05) [8] . Please note, if an allele occurs in a population with a MAF of more than 1% (0.01), it means that a considerable number of individuals carry this allele, and it is very unlikely to cause disease (ExAC).
7. Quality- QUAL scores are transformed log-scaled (PHRED) values where, for example, a score of 90 supports the variant call with a P-value of 1x10-9[9]. Qual >30 is acceptable (p value -1 x10-3. 99.9%) [10]
8. Depth of read (VCF format)- number of reads which passed the internal quality control metrics (after filtering) – above 100 reads is considered to be acceptable [11, 12] 9. Please note coverage of each sample was checked according to set threshold by TSU500 app guidelines.

When using NGS for clinical diagnostics, multiple observations for a single base are necessary for a reliable variant calling. There are no official guidelines due to the analysis being influenced by numerous factors such as: the length of the reads, the size of the reference genome, the specific application of interest, the error rate of the technology used, the genes expression level and the complexity of the target regions. We have used the following parameters to call driver mutations using the Illumina TruSight Oncology 500 (TSO500) panel [8] carried out by the Southwest genomic hub.

Quality criteria	Value
FATHMM_MKL Prediction	D=damaging
QUALITY score	>30
Variant allele frequency	greater or = 0.05
Minor allele frequency	less than 0.05
Depth (DP VCF)	>100
Gene Coverage (50X) |	>90%

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