For Research Use Only

# AKT Polyclonal antibody

Catalog Number:10176-2-AP Featured Product 998 Publications

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## Basic Information

Catalog Number:
10176-2-AP

Size:
150ul , Concentration: 600 μg/ml by Nanodrop;

Source:
Rabbit

Isotype:
IgG

Immunogen Catalog Number:
AG0213

GenBank Accession Number:
BC000479

GeneID (NCBI):
207

Full Name:
v-akt murine thymoma viral oncogene homolog 1

Calculated MW:
56 kDa

Observed MW:
56-62 kDa

Purification Method:
Antigen affinity purification

Recommended Dilutions:
WB 1:2000-1:12000
IP 0.5-4.0 ug for IP and 1:500-1:1000 for WB
IHC 1:50-1:500
IF 1:50-1:500

## Applications

Tested Applications:
FC, IF, IHC, IP, WB, ELISA

Cited Applications:
CoIP, IF, IHC, IP, WB

Species Specificity:
human, mouse, rat

Cited Species:
human, goat, chicken, rat, zebra finches, mouse, fish, Zebrafish, hamster, pig

***Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0***

Positive Controls:

WB : A549 cells, HeLa cells, HepG2 cells, MCF-7 cells, NIH/3T3 cells, C6 cells, mouse brain tissue, mouse liver tissue, rat brain tissue

IP : HeLa cells,

IHC : human ovary tumor tissue, human breast cancer tissue

IF : HeLa cells, mouse brain tissue

## Background Information

The serine-threonine protein kinase AKT1 is catalytically inactive in serum-starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of AKT1. It was shown that the activation occurs through phosphatidylinositol 3-kinase. In the developing nervous system AKT is a critical mediator of growth factor-induced neuronal survival. Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Yangmeng Zhao | 36178125 | Redox Rep | WB |
| Xiao-Feng Zhu | 36180975 | Phytother Res | WB |
| Tong Li | 33152931 | Biomed Pharmacother | WB |

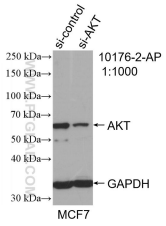
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

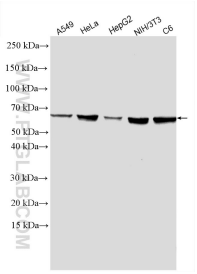
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

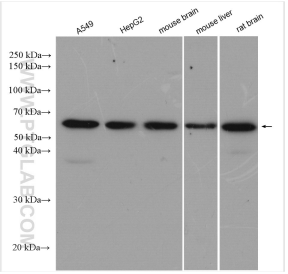
## Selected Validation Data



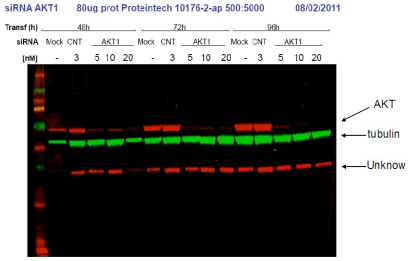

WB result of AKT antibody (10176-2-AP; 1:1000; incubated at room temperature for 1.5 hours) with sh-Control and sh-AKT transfected MCF-7 cells.



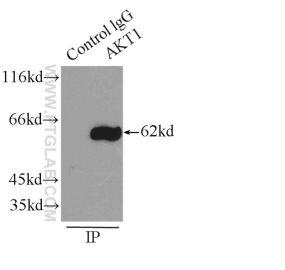

Various lysates were subjected to SDS PAGE followed by western blot with 10176-2-AP (AKT antibody) at dilution of 1:4000 incubated at room temperature for 1.5 hours.



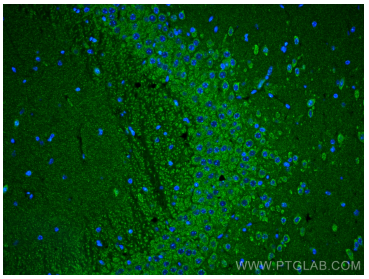

Various lysates were subjected to SDS PAGE followed by western blot with 10176-2-AP (AKT antibody) at dilution of 1:6000 incubated at room temperature for 1.5 hours.



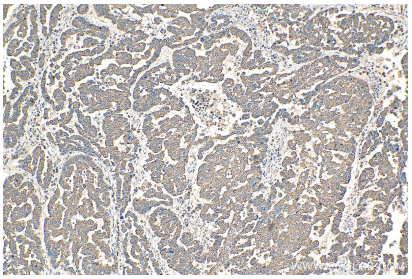

siRNA AKT1 result from Dr. Eva Martinez-Balibrea. Green:tubulin, Red:10176-2-AP, AKT1.



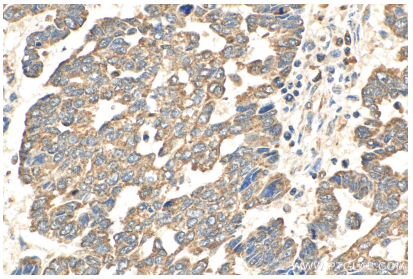

IP Result of anti-AKT1 (IP:10176-2-AP, 3ug; Detection:10176-2-AP 1:500) with HeLa cells lysate 2500ug.



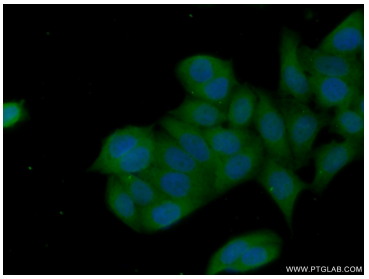

Immunofluorescent analysis of (4% PFA) fixed mouse brain tissue using AKT antibody (10176-2-AP) at dilution of 1:400 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).



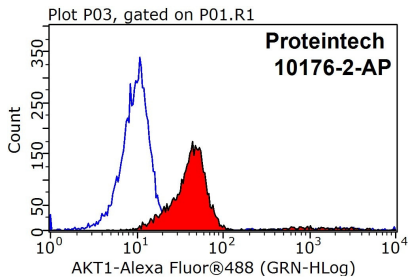
Immunohistochemical analysis of paraffin-embedded human ovary tumor tissue slide using 10176-2-AP (AKT antibody) at dilution of 1:200 (under 10x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).



Immunohistochemical analysis of paraffin-embedded human ovary tumor tissue slide using 10176-2-AP (AKT antibody) at dilution of 1:200 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).




Immunofluorescent analysis of (10% Formaldehyde) fixed HeLa cells using 10176-2-AP (AKT1 antibody) at dilution of 1:50 and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).




1X10^6 HeLa cells were stained with 0.2ug AKT1 antibody (10176-2-AP, red) and control antibody (blue). Fixed with 90% MeOH blocked with 3% BSA (30 min). Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) with dilution 1:1000.

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# Catalase Polyclonal antibody

Catalog Number:21260-1-AP 120 Publications

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## Basic Information

Catalog Number:
21260-1-AP

Size:
150ul , Concentration: 350 μg/ml by Nanodrop;

Source:
Rabbit

Isotype:
IgG

Immunogen Catalog Number:
AG15383

GenBank Accession Number:
BC112219

GeneID (NCBI):
847

Full Name:
catalase

Calculated MW:
60 kDa

Observed MW:
60 kDa

Purification Method:
Antigen affinity purification

Recommended Dilutions:
WB 1:2000-1:16000
IHC 1:250-1:1000
IF 1:10-1:100

## Applications

Tested Applications:
IF, IHC, WB, ELISA

Cited Applications:
FC, IF, IHC, WB

Species Specificity:
human, mouse, rat

Cited Species:
Meretrix meretrix, human, goat, rat, sheep, mouse, pig, bat, bovine, Fungus

***Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0***

Positive Controls:

WB : HeLa cells, Jurkat cells, Raji cells, rat liver tissue, HepG2 cells, L02 cells, mouse liver tissue

IHC : human liver cancer tissue, human hepatocirrhosis tissue

IF : HepG2 cells, HeLa cells

## Background Information

Catalase belongs to the catalase family. CAT occurs in almost all aerobically respiring organisms and serves to protect cells from the toxic effects of hydrogen peroxide. CAT promotes growth of cells including T-cells, B-cells, myeloid leukemia cells, melanoma cells, mastocytoma cells and normal and transformed fibroblast cells. CAT catalyzes the reaction: 2 H2O2 = O2 + 2 H2O. Defects in CAT are the cause of acatalasia (ACATLAS) which also known as acatalasemia.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Wei Cai | 36170234 | Autophagy | IF |
| Lin-Yu Jin | 36164394 | Oxid Med Cell Longev | WB |
| Hong Zou | 36139126 | Biomolecules | WB |

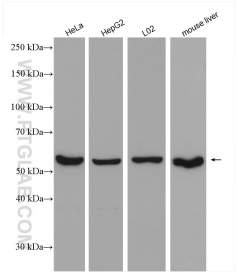
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

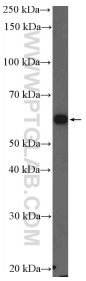
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

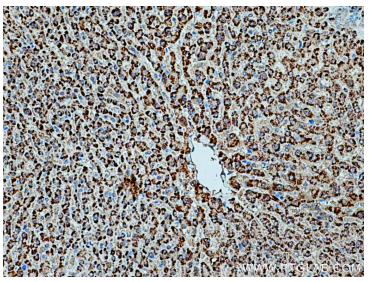
## Selected Validation Data



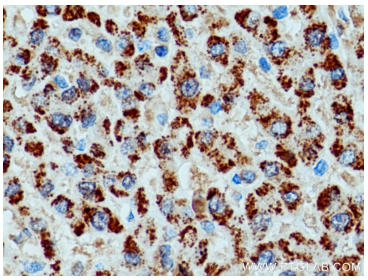

Various lysates were subjected to SDS PAGE followed by western blot with 21260-1-AP (Catalase antibody) at dilution of 1:8000 incubated at room temperature for 1.5 hours.



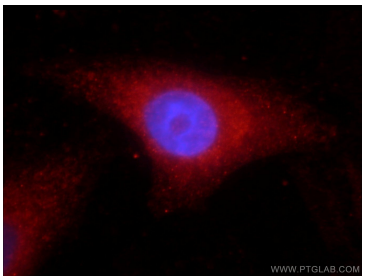

Jurkat cells were subjected to SDS PAGE followed by western blot with 21260-1-AP (Catalase Antibody) at dilution of 1:1000 incubated at room temperature for 1.5 hours.



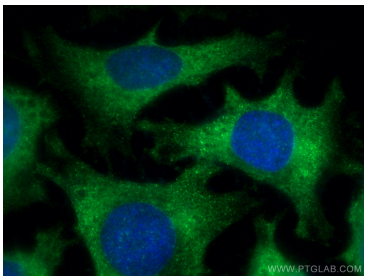
Immunohistochemical analysis of paraffin-embedded human liver cancer tissue slide using 21260-1-AP (Catalase antibody) at dilution of 1:500 (under 10x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).



Immunohistochemical analysis of paraffin-embedded human liver cancer tissue slide using 21260-1-AP (Catalase antibody) at dilution of 1:500 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).




Immunofluorescent analysis of HepG2 cells using 21260-1-AP (Catalase antibody) at dilution of 1:25 and Rhodamine-Goat anti-Rabbit IgG.




Immunofluorescent analysis of (-20°C Methanol) fixed HeLa cells using Catalase antibody (21260-1-AP) at dilution of 1:100 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).

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# Collagen Type III (N-terminal) Polyclonal antibody

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Catalog Number:22734-1-AP Featured Product 344 Publications

## Basic Information

Catalog Number:
22734-1-AP

Size:
150ul , Concentration: 900 μg/ml by Nanodrop;

Source:
Rabbit

Isotype:
IgG

Immunogen Catalog Number:
AG18658

GenBank Accession Number:
BC028178

GeneID (NCBI):
1281

Full Name:
collagen, type III, alpha 1

Calculated MW:
1466 aa, 139 kDa

Observed MW:
140-180 kDa

Purification Method:
Antigen affinity purification

Recommended Dilutions:
WB 1:300-1:1000
IP 0.5-4.0 ug for IP and 1:500-1:1000 for WB
IHC 1:500-1:2000
IF 1:50-1:500

## Applications

Tested Applications:
IF, IHC, IP, WB, ELISA

Cited Applications:
IF, IHC, WB

Species Specificity:
human, mouse, rat

Cited Species:
human, rat, mouse, rabbit, hamster, pig, canine

***Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0***

Positive Controls:

WB : mouse skin tissue, rat skin tissue

IP : mouse skin tissue,

IHC : human hepatocirrhosis tissue, human pancreas cancer tissue, mouse liver tissue, human skin cancer tissue, mouse heart tissue, mouse kidney tissue, human colon tissue, mouse colon tissue, human skin tissue, human malignant melanoma tissue

IF : human colon tissue,

## Background Information

Type III collagen is a fibrillar forming collagen comprising three α1(III) chains and is expressed in early embryos and throughout embryogenesis (PMID: 9050868). In the adult, type III collagen is a major component of the extracellular matrix in a variety of internal organs and skin. It occurs in most soft connective tissues along with type I collagen (PMID: 2445760). COL3A1 gene encodes type III procollagen. Mutations in this gene are associated with Ehlers-Danlos syndrome types IV, and with aortic and arterial aneurysms (PMID: 10706896; 2243125; 18389341). This antibody raised against 24-152 aa of prepro α1 (III) chain of human type III procollagen detects type III procollagen at 140-180 kDa and also in some lysates reveals a 70-kDa band which has been reported and may represent a cleaved form of type III procollagen (PMID: 17424834; 19648160; 22802960).

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Lexun Wang | 34621323 | Evid Based Complement Alternat Med | WB |
| Dandan Zhang | 36225554 | Front Pharmacol | WB |
| Yu Sun | 34562065 | J Cell Mol Med | WB |

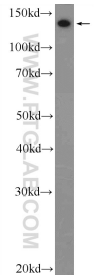
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

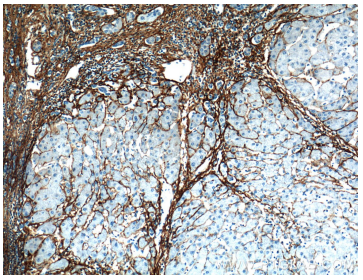
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

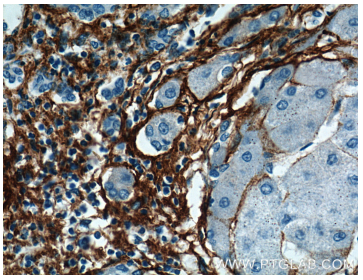
## Selected Validation Data



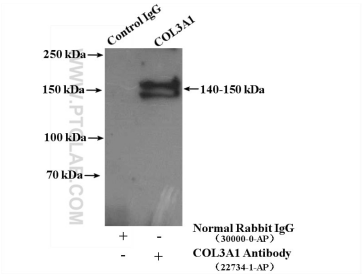

mouse skin tissue were subjected to SDS PAGE followed by western blot with 22734-1-AP (Collagen Type III (N-terminal) antibody at dilution of 1:1000 incubated at room temperature for 1.5 hours.



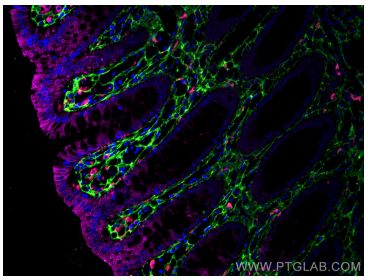
Immunohistochemical analysis of paraffin-embedded human hepatocirrhosis tissue slide using 22734-1-AP (Collagen Type III (N-terminal) antibody at dilution of 1:1000 (under 10x lens).




Immunohistochemical analysis of paraffin-embedded human hepatocirrhosis tissue slide using 22734-1-AP (Collagen Type III (N-terminal) antibody at dilution of 1:1000 (under 40x lens).




IP Result of anti-Collagen Type III (N-terminal) (IP:22734-1-AP, 4ug; Detection:22734-1-AP 1:500) with mouse skin tissue lysate 3200ug.




Immunofluorescent analysis of (4% PFA) fixed human colon tissue using Collagen Type III (N-terminal) antibody (22734-1-AP) at dilution of 1:200 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L), Cytokeratin 20 antibody (60183-1-Ig, Clone: 4D10A4, Magenta), CoraLite®594 CD68 antibody (CL594-25747, red).

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# GAPDH Monoclonal antibody

Catalog Number:60004-1-Ig Featured Product 8979 Publications

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## Basic Information

Catalog Number:
60004-1-Ig

Size:
150ul , Concentration: 1000 μg/ml by Nanodrop;

Source:
Mouse

Isotype:
IgG2b

Immunogen Catalog Number:
AG0766

GenBank Accession Number:
BC004109

GeneID (NCBI):
2597

Full Name:
glyceraldehyde-3-phosphate dehydrogenase

Calculated MW:
36 kDa

Observed MW:
36 kDa

Purification Method:
Protein A purification

CloneNo.:
1E6D9

Recommended Dilutions:
WB 1:50000-1:500000
IP 0.5-4.0 ug for IP and 1:2000-1:12000 for WB
IF 1:200-1:2000
FC

## Applications

Tested Applications:
FC, IF, IP, WB, ELISA

Cited Applications:
Cell treatment, CoIP, FC, IF, IHC, IP, WB

Species Specificity:
human, mouse, rat, yeast, plant, zebrafish

Cited Species:
Deer, human, Goat, tick, treeshrew, Cynomorium songaricum, chicken, whiteflies, Yeast, rat

Positive Controls:

WB : HeLa cells, HepG2 cells, ROS1728 cells, pig brain tissue, zebrafish tissue, whole yeast, whole Nematode tissue, soybean whole plant tissue, arabidopsis whole plant tissue, HEK-293 cells, Jurkat cells, K-562 cells, HSC-T6 cells, NIH/3T3 cells, 4T1 cells, C6 cells, PC-12 cells, C2C12 cells, SP2/0 cells, rat brain tissue, mouse brain tissue

IP : HeLa Cells,

IF : Ethacrynic acid treated HeLa cells,

FC : HeLa cells,

## Background Information

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis. GAPDH participates in nuclear events including transcription, binding RNA, RNA transportation, DNA replication, DNA repair and apoptosis. Being stably and constitutively expressed at high levels in most tissues and cells, GAPDH is considered a housekeeping protein. It is widely used as a control for RT-PCR and also loading control in electrophoresis and Western blotting. GAPDH is normally expressed in cellular cytoplasm or membrane, but can occasionally translocate to the nucleus after the addition of post-translational modifications such as S-nitrosylation. This antibody is raised against full length GAPDH of human origin. It can recognize the 36 kDa GAPDH protein in most cells/tissues. In addition, a band below 36 kDa can always be detected as the isoform or spliced product of GAPDH (PMID: 23885286, 23877755, 19368702). Please note that some physiological factors, such as hypoxia and diabetes, increase GAPDH expression in certain cell types. For murine tissue samples, conjugated mouse antibody HRP-60004 and rabbit antibody 10494-1-AP are preferable.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Yuying Wang | 36183783 | Chem Biol Interact | WB |
| Xin Shen | 36184549 | Int Heart J | WB |
| Yueke Lin | 36178239 | EMBO Rep | WB,IP |

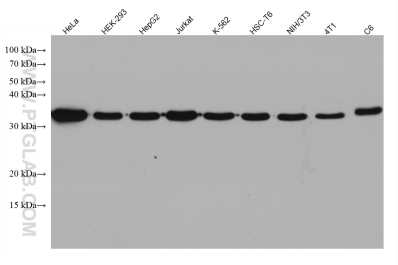
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

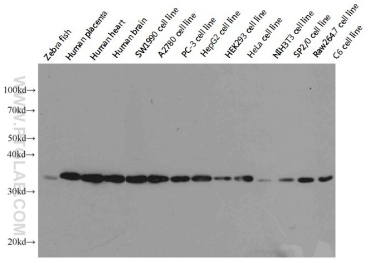
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

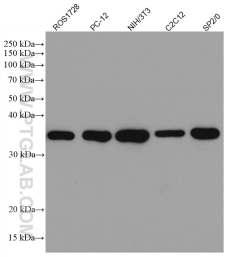
## Selected Validation Data



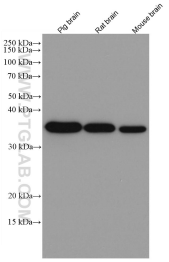

Various lysates were subjected to SDS PAGE followed by western blot with 60004-1-Ig (GAPDH antibody) at dilution of 1:200000 incubated at room temperature for 1.5 hours.



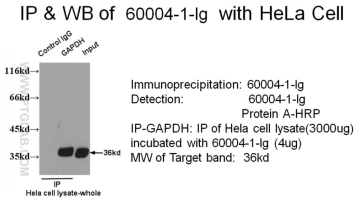

Western blot analysis of GAPDH in various tissues and cell lines using Proteintech antibody 60004-1-Ig at a dilution of 1:10000.



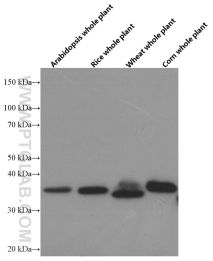

Various lysates were subjected to SDS PAGE followed by western blot with 60004-1-Ig (GAPDH antibody) at dilution of 1:50000 incubated at room temperature for 1.5 hours.



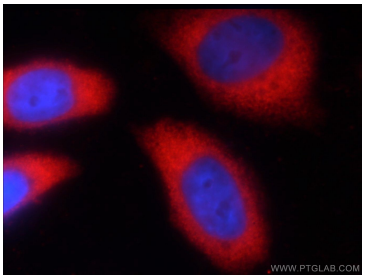

Various lysates were subjected to SDS PAGE followed by western blot with 60004-1-Ig (GAPDH antibody) at dilution of 1:50000 incubated at room temperature for 1.5 hours.



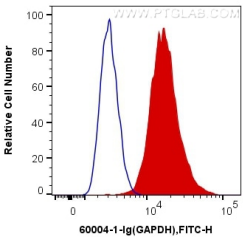

IP result of anti-GAPDH (60004-1-Ig for IP and Detection) with HeLa cell lysate.




arabidopsis, rice, wheat, corn whole plant tissue were subjected to SDS PAGE followed by western blot with 60004-1-Ig (GAPDH Antibody) at dilution of 1:10000 incubated at room temperature for 1.5 hours.




Immunofluorescent analysis of EA treated HeLa cells using 60004-1-Ig(GAPDH antibody) at dilution of 1:50 and Rhodamine-labeled goat anti-mouse IgG (red).




1X10^6 HeLa cells were intracellularly stained with 0.4 ug Anti-Human GAPDH (60004-1-Ig, Clone:1E6D9) and CoraLite®488-Conjugated AffiniPure Goat Anti-Mouse IgG(H+L) at dilution 1:1000 (red), or 0.4 ug Mouse IgG2b Isotype Control (66360-3-Ig, Clone: K11B8C4B5) (blue). Cells were fixed with 4% PFA and permeabilized with Flow Cytometry Perm Buffer (PF00011-C).

For Research Use Only

# NF-κB p65 Polyclonal antibody

Catalog Number:10745-1-AP Featured Product 1101 Publications

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## Basic Information

Catalog Number:
10745-1-AP

Size:
150ul , Concentration: 520 μg/ml by Nanodrop;

Source:
Rabbit

Isotype:
IgG

Immunogen Catalog Number:
AG1199

GenBank Accession Number:
BC011603

GeneID (NCBI):
5970

Full Name:
v-rel reticuloendotheliosis viral oncogene homolog A (avian)

Calculated MW:
65 kDa

Observed MW:
65 kDa

Purification Method:
Antigen affinity purification

Recommended Dilutions:
WB 1:1000-1:6000
IP 0.5-4.0 ug for IP and 1:500-1:2000 for WB
IHC 1:50-1:500
IF 1:50-1:500

## Applications

Tested Applications:
FC, IF, IHC, IP, WB, ELISA

Cited Applications:
Cell treatment, ChIP, CoIP, ELISA, IF, IHC, IP, WB

Species Specificity:
human, mouse, rat

Cited Species:
human, goat, CHICKEN, rat, mouse, monkey, rabbit, fish, hamster, pig

***Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0***

Positive Controls:

WB : A431 cells, Hek-293 cells, Jurkat cells, MCF-7 cells, K-562 cells, HeLa cells, Raji cells, NIH/3T3 cells

IP : HeLa cells,

IHC : human breast cancer tissue, human stomach tissue, human liver cancer tissue

IF : HepG2 cells,

## Background Information

Nuclear factor k B (NF-kB) is a sequence-specific DNA-binding protein complex which regulates the expression of viral genomes, including the human immunodeficiency virus, and a variety of cellular genes, particularly those involved in immune and inflammatory responses. The members of the NF-kB family in mammalian cells include the proto-oncogene c-Rel,p50/p105 (NFkB1), p65 (RelA), p52/p100 (NFkB2), and RelB. All of these proteins share a conserved 300-amino acid region known as the Rel homology domain which is responsible for DNA binding, dimerization, and nuclear translocation of NF-kB. The p65 subunit is a major component of NF-kB complexes and is responsible for trans-activation. NF-kB heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-kB p65-p65 complex appears to be involved in invasin-mediated activation of IL-8 expression. The inhibitory effect of IkB upon NF-kB the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kB complex. It associates with chromatin at the NF-kB promoter region via association with DDX1. This antibody is a rabbit polyclonal antibody raised against residues near the N terminus of human RELA.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Ji Xing | 36230734 | Cancers (Basel) | WB |
| Chang Liu | 36230117 | Foods | WB |
| Yanliang Wu | 34601083 | J Ethnopharmacol | WB |

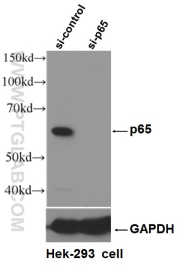
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

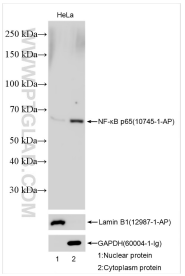
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

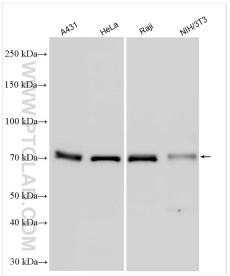
## Selected Validation Data



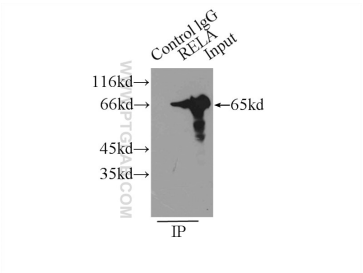

WB result of RELA, p65antibody (10745-1-AP, 1:500) with si-Control and si-RELA,p65 transfected HeLa cells.



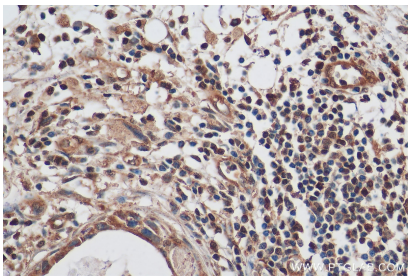

HeLa nuclear fraction and cytoplasmic fraction were subjected to SDS PAGE saparately, followed by western blot with 10745-1-AP (NF-κB p65 antibody) at dilution of 1:6000 incubated at room temperature for 1.5 hours. The membrane was stripped, reblotted with lamin B1 (12987-1-AP) & GAPDH (60004-1-Ig) antibodies as the loading control.



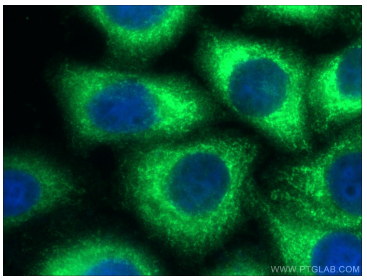

Various lysates were subjected to SDS PAGE followed by western blot with 10745-1-AP (NF-κB p65 antibody) at dilution of 1:3000 incubated at room temperature for 1.5 hours.



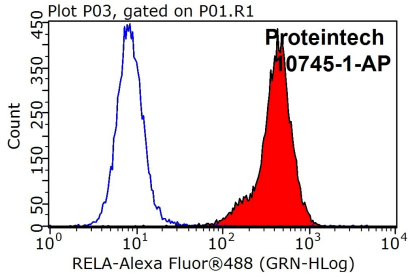

IP Result of anti-p65 (IP:10745-1-AP, 3ug; Detection:10745-1-AP 1:1000) with HeLa cells lysate 5000ug.



Immunohistochemical analysis of paraffin-embedded human breast cancer tissue slide using 10745-1-AP (NF-κB p65 antibody) at dilution of 1:200 (under 0x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).



Immunofluorescent analysis of (-20°C Ethanol) fixed HepG2 cells using 10745-1-AP (p65; RELA antibody) at dilution of 1:100 and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).




1X10^6 HeLa cells were stained with 0.2ug p65; RELA antibody (10745-1-AP, red) and control antibody (blue). Fixed with 90% MeOH blocked with 3% BSA (30 min). Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) with dilution 1:1000.

For Research Use Only

# Phospho-AKT (Ser473) Monoclonal antibody

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Catalog Number:66444-1-Ig

801 Publications

## Basic Information

Catalog Number:
66444-1-Ig

Size:
100ul , Concentration: 1500 μg/ml by Nanodrop;

Source:
Mouse

Isotype:
IgG1

GenBank Accession Number:
NM_005163

GeneID (NCBI):
207

Full Name:
v-akt murine thymoma viral oncogene homolog 1

Observed MW:
60-62 kDa

Purification Method:
Protein A purification

CloneNo.:
1C10B8

Recommended Dilutions:
WB 1:5000-1:50000
IHC 1:100-1:400

## Applications

Tested Applications:
FC, IHC, WB, ELISA

Cited Applications:
FC, IF, IHC, WB

Species Specificity:
human, mouse, rat

Cited Species:
human, chicken, rat, mouse, rabbit, zebrafish, pig, canine

***Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0***

Positive Controls:

WB : Calyculin A treated Jurkat cells, Calyculin A treated HEK-293T cells, Calyculin A treated PC-3 cells, Calyculin A treated HEK-293 cells, TPA treated Jurkat cells, Calyculin A treated HSC-T6 cells, Calyculin A treated NIH/3T3 cells

IHC : human breast cancer tissue, Calyculin A treated Jurkat cells, human colon cancer tissue

## Background Information

The serine-threonine protein kinase AKT1 is catalytically inactive in serum-starved primary and immortalized fibroblasts. Survival factors can suppress apoptosis in a transcription-independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery. This antibody detects all the members of AKT with phospho-modification at Ser473.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Wenzhong Peng | 36274350 | Tissue Cell | WB |
| Tong Li | 33152931 | Biomed Pharmacother | WB |
| Di Cui | 36175877 | BMC Cancer | WB |

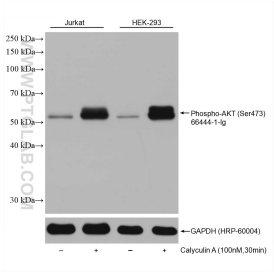
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

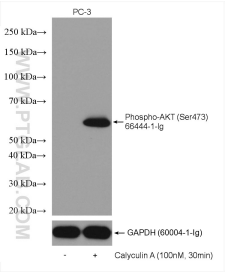
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

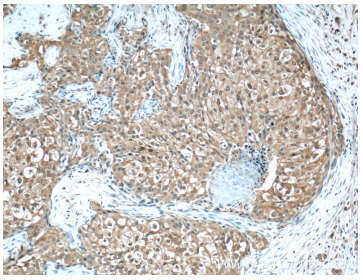
## Selected Validation Data



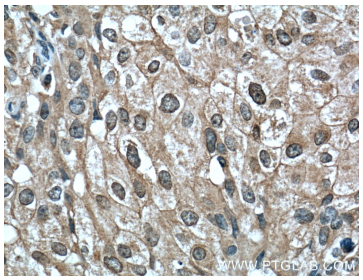

Non-treated Jurkat and HEK-293 cells and Calyculin A treated Jurkat and HEK-293 cells were subjected to SDS PAGE followed by western blot with 66444-1-Ig (Phospho-AKT (Ser473) antibody) at dilution of 1:10000 incubated at room temperature for 1.5 hours.



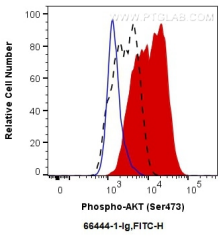

Non-treated PC-3 and Calyculin A treated PC-3 cells were subjected to SDS PAGE followed by western blot with 66444-1-Ig (Phospho-AKT (Ser473) antibody) at dilution of 1:5000 incubated at room temperature for 1.5 hours. The membrane was stripped and re-blotted with GAPDH antibody as loading control.



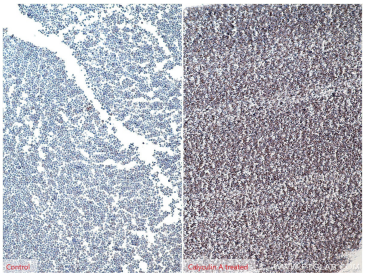
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue slide using 66444-1-Ig (AKT-phospho-S473 antibody at dilution of 1:200 (under 10x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).



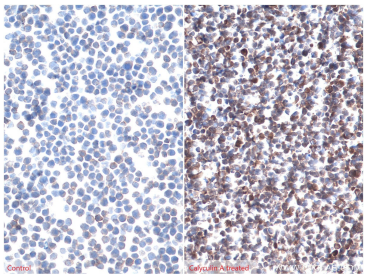

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue slide using 66444-1-Ig (AKT-phospho-S473 antibody at dilution of 1:200 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).




1X10^6 PC-3 cells untreated (dashed line) or treated with Calyculin A (red) were intracellularly stained with 0.5 ug Anti-Human Phospho-AKT (Ser473) (66444-1-Ig, Clone:1C10B8) and CoraLite®488-Conjugated AffiniPure Goat Anti-Mouse IgG(H+L) at dilution 1:1000, or 0.5 ug Control Antibody (blue). Cells were fixed with 4% PFA and permeabilized with 90% MeOH.




Immunohistochemical analysis of paraffin-embedded untreated (left) or Calyculin A treated (right) Jurkat cells slide using 66444-1-Ig (Phospho-AKT (Ser473) antibody) at dilution of 1:8000 (under 10x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).




Immunohistochemical analysis of paraffin-embedded untreated (left) or Calyculin A treated (right) Jurkat cells slide using 66444-1-Ig (Phospho-AKT (Ser473) antibody) at dilution of 1:8000 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).

For Research Use Only

# Phospho-NF-κB p65 (Ser468) Recombinant antibody

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Catalog Number:82335-1-RR 11 Publications

## Basic Information

Catalog Number:
82335-1-RR

Size:
100ul , Concentration: 500 μg/ml by Nanodrop;

Source:
Rabbit

Isotype:
IgG

GenBank Accession Number:
BC011603

GeneID (NCBI):
5970

Full Name:
v-rel reticuloendotheliosis viral oncogene homolog A (avian)

Calculated MW:
65 kDa

Observed MW:
75 kDa

Purification Method:
Protein A purification

CloneNo.:
6N1

Recommended Dilutions:
WB 1:2000-1:10000

## Applications

Tested Applications:
WB, ELISA

Cited Applications:
WB

Species Specificity:
Human, Mouse

Cited Species:
human, rat, mouse, pig

Positive Controls:

WB : Calyculin A treated HeLa cells, Calyculin A treated NIH/3T3 cells

## Background Information

Nuclear factor kB (NF-kB) is a collective term for a small family of dimeric transcription factors [comprising p65 (RelA) and RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2)]. All NF-κB proteins share a Rel homology domain (RHD), which is responsible for DNA binding and dimerization. Only p65, RelB, and c-Rel contain potent transactivation domains within sequences from the C-terminal to the RHD. Exterior signals lead to the phosphorylation and degradation of the inhibitory complex IκB, which is modulated by the IκB kinase (IKK), and its degradation allows for the release of the typical NF-κB heterodimer, p65/p50, to translocate into the nucleus. NF-κB binds to its cognate DNA elements and can transcriptionally activate different target genes among which 200-500 genes have been implicated in cell survival/apoptosis, cell growth, immune response, and inflammation.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Ruiyu Zhou | 37822185 | Brain Behav | WB |
| Hong-Gang Wang | 37716259 | Transl Oncol | WB |
| Meng Meng | 37586160 | Phytomedicine | WB |

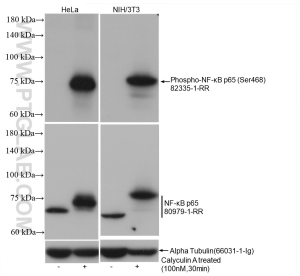
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

## Selected Validation Data




Non-treated and Calyculin A treated various cells were subjected to SDS PAGE followed by western blot with 82335-1-RR (Phospho-NF-κB p65 (Ser468) antibody) at dilution of 1:5000 incubated at room temperature for 1.5 hours. The membrane was stripped and reblotted with Alpha Tubulin antibody (66031-1-Ig) and NF-κB p65 antibody (80979-1-RR) subsequently.

For Research Use Only

# Beta Actin Monoclonal antibody

Catalog Number:66009-1-Ig Featured Product 4926 Publications

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## Basic Information

Catalog Number:
66009-1-Ig

Size:
150ul , Concentration: 1000 μg/ml by Nanodrop;

Source:
Mouse

Isotype:
IgG2b

GenBank Accession Number:
NM_001101

GeneID (NCBI):
60

Full Name:
actin, beta

Calculated MW:
42 kDa

Observed MW:
42 kDa

Purification Method:
Protein A purification

CloneNo.:
2D4H5

Recommended Dilutions:
WB 1:20000-1:100000
IP 0.5-4.0 ug for IP and 1:5000-1:50000 for WB
IHC 1:20-1:2000
IF 1:500-1:2000

## Applications

Tested Applications:
FC, IF, IHC, IP, WB, ELISA

Cited Applications:
Cell treatment, ChIP, CoIP, ELISA, IF, IHC, IP, WB

Species Specificity:
human, mouse, rat, hamster, monkey, dog, pig, chicken, rabbit, zebrafish

Cited Species:
human, goat, chicken, rat, Arabidopsis, Golden hamsters, Aedes albopictus, mouse, monkey, rabbit

***Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0***

Positive Controls:

WB : HeLa cells, A549 cells, Jurkat cells, HSC-T6 cells. NIH/3T3 cells, Pig brain, Rabbit brain, Rat brain, Mouse brain, Chicken brain, HEK-293 cells, HepG2 cells, K-562 cells, HHSC-T6 cells, 4T1 cells, CHO cells, mouse pancreas, rat pancreas

IP : HeLa cells,

IHC : human kidney tissue, human brain tissue, human colon cancer tissue, human heart tissue

IF : MDCK cells, HeLa cells

## Background Information

Actins are highly conserved globular proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. At least six isoforms of actins are known in mammals and other vertebrates: alpha (ACTC1, cardiac muscle 1), alpha 1 (ACTA1, skeletal muscle) and 2 (ACTA2, aortic smooth muscle), beta (ACTB), gamma 1 (ACTG1) and 2 (ACTG2, enteric smooth muscle). Beta and gamma 1 are two non-muscle actin proteins. Most actins consist of 376aa, while ACTG2 (rich in muscles) has 375aa and ACTG1(found in non-muscle cells) has only 374aa. Beta actin has been widely used as the internal control in RT-PCR and Western Blotting as a 42-kDa protein. However, the 41 kDa cleaved fragment of beta actin can be generated during apoptosis process.This antibody can recognize all the actins. The isotype of this antibody is IgG2b. For murine tissue sample, conjugated antibody (HRP-66009) or rabbit antibody (20536-1-AP) is preferable.

## Notable Publications

| Author | Pubmed ID | Journal | Application |
|---|---|---|---|
| Xiaoxuan Zhai | 36184541 | Int Heart J | WB |
| Yueke Lin | 36178239 | EMBO Rep | WB |
| Yangfei Yi | 36178080 | Orthop Surg | WB |

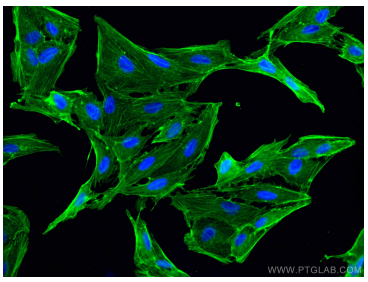
## Storage

Storage:
Store at -20°C. Stable for one year after shipment.
Storage Buffer:
PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
Aliquoting is unnecessary for -20°C storage

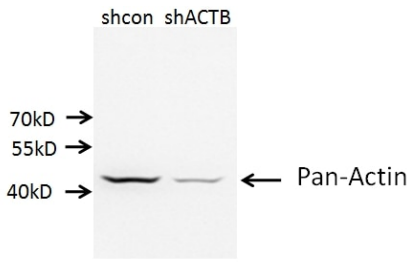
*** 20ul sizes contain 0.1% BSA

For technical support and original validation data for this product please contact:
T: 1 (888) 4PTGLAB (1-888-478-4522) (toll free in USA), or 1(312) 455-8498 (outside USA)
E: proteintech@ptglab.com
W: ptglab.com

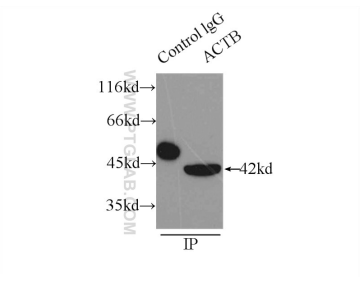
## Selected Validation Data



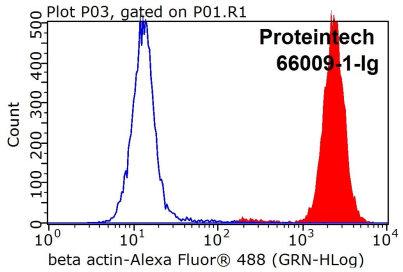
Immunofluorescent analysis of (-20°C Methanol) fixed MDCK cells using Beta Actin antibody (66009-1-Ig, Clone: 2D4H5 ) at dilution of 1:1000 and CoraLite®488-Conjugated AffiniPure Goat Anti-Mouse IgG(H+L).



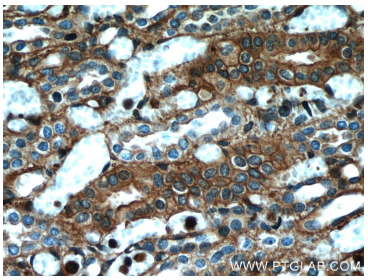

A549 cells (shcontrol and shRNA of Beta Actin) were subjected to SDS PAGE followed by western blot with 66009-1-Ig (Mouse anti Pan-actin antibody) at dilution of 1:10000.



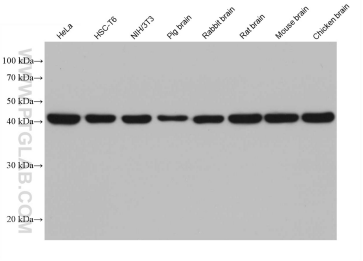

IP Result of anti-beta actin (IP:66009-1-Ig, 3ug; Detection:66009-1-Ig 1:10000) with HeLa cells lysate 3100ug.



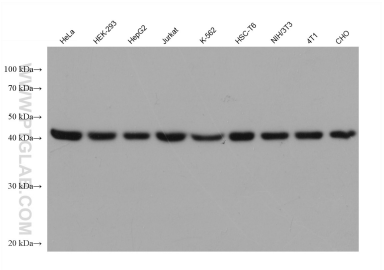

1X10^6 HeLa cells were stained with 0.05 ug Anti-Human Beta Actin (66009-1-Ig, Clone:2D4H5) and FITC-Goat anti-mouse IgG at dilution 1:100 (red), or stained with 0.05 ug isotype control and FITC-Goat anti-mouse IgG at dilution 1:100 (blue). Cells were fixed with 4% PFA and permeabilized with 0.1% TritonX-100.



Immunohistochemical analysis of paraffin-embedded human kidney using 66009-1-Ig at dilution of 1:500 (under 40x lens).




Various lysates were subjected to SDS PAGE followed by western blot with 66009-1-Ig (Beta Actin antibody) at dilution of 1:100000 incubated at room temperature for 1.5 hours.




Various lysates were subjected to SDS PAGE followed by western blot with 66009-1-Ig (Beta Actin antibody) at dilution of 1:200000 incubated at room temperature for 1.5 hours.

# Reactive Oxygen Species (ROS)
# Fluorometric Assay Kit

Catalog No: E-BC-K138-F

Method: Fluorimetric method

Specification: 96T

Instrument: Fluorescence Microplate reader, Flow Cytometry

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

# General information

## ▲Intended use

This kit can be used to measure reactive oxygen species (ROS) in fresh tissue and cell samples.

## ▲Background

Reactive oxygen species (ROS) are active chemical substances produced in the metabolic process of the body, including oxygen free radicals, hydrogen peroxide and its downstream products, such as peroxides and hydroxides. ROS are both necessary and harmful to organisms, and are involved in cell growth, proliferation, development and differentiation, aging and apoptosis, as well as many physiological and pathological processes. Excessive ROS will lead to oxidative stress and oxidative damage of cells, further promoting the occurrence and development of many diseases, such as cancer, cardiovascular disease and diabetes.

## ▲Detection principle

DCFH-DA (2,7-dichlorofuorescin diacetate) is a fluorescent probe without fluorescence that can freely cross the membrane. After entering the cell, it can be hydrolyzed by intracellular esterase to form DCFH (dichlorofluorescin). In the presence of reactive oxygen species (ROS), DCFH is oxidized to DCF (dichlorofluorescein) which is a strong green fluorescent substance that cannot penetrate the cell membrane. DCF has a maximum wave peak near the excitation wavelength of 502 nm and the emission wavelength of 525 nm, and the intensity is proportional to the level of intracellular reactive oxygen species.

## ▲Kit components & storage

| Item | Component | Specification | Storage |
|---|---|---|---|
| Reagent 1 | 10 mmol/L DCFH-DA | 0.1 mL × 1 vial | -20℃ , 12 months, shading light |
| Reagent 2 | Positive Control | 1 mL × 1 vial | 2-8℃ , 12 months |
| | Black Microplate | 96 wells × 2 | No requirement |
| | Plate Sealer | 4 pieces | |
| Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. | | | |

## ▲Materials prepared by users

### Instruments

Fluorescence Microplate reader (Ex/Em=500 nm/525 nm), Flow Cytometry(Ex/Em=500 nm/525 nm), Vortex mixer, Micropipettor, Water bath, Incubator, Centrifuge

### Reagents

Double distilled water、PBS（0.01 M，pH 7.4）、Serum-free medium

## ▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

## ▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

## ▲The key points of the assay

1. After the incubation of the probe, it is important to wash out residual probes that have not entered the cells, otherwise the background will be higher.
2. Avoid repeated freezing and thawing of DCFH-DA.
3. The time of detection is shortened as far as possible to reduce the experimental error.
4. Set a positive control (reagent 2 working solution) and a negative control (only cells without reagent 1 working solution).

## Pre-assay preparation

1. Preparation of reagent 1 working solution

Dilute the reagent 1 with serum-free medium, the recommended working concentration is 0.1-20 μM. Prepare the fresh solution before use.

(Note: DMSO is harmful to cells, so the dilution ratio must be more than 500.)

2. Preparation of reagent 2 working solution

Dilute the reagent 2 (contain 10 mM TBHP) with serum-free medium, the recommended working concentration of TBHP is 50-250 μM. Prepare the fresh solution before use.

# Assay protocol

## ▲Detection of culture cell sample

### 1. Add the fluorescent probe:

a. Add Reagent 1 working solution to the cells. The DCFH-DA working concentration can be 0.1-20 μM for different cells and treatment. Pre-experiment is suggested to determine the appropriate concentration. The total dilution ratio should be more than 1:500-1:1000 in order to avoid effects of DMSO on cells. DMSO should be set as solution control.

b. Incubate at 37℃ for 30 min ~ few hours, generally 30~60 min. The incubation time is related to cell types, stimulation conditions, and DCFH-DA concentration.

c. Cell collection:

Suspension cells: centrifuge the sample at 1000 g for 5~10 min and wash with serum-free medium for 2~3 times. Centrifuge and collect the cell precipitation for fluorescence detection.

Adherent cells: digest the cells with 0.25% trypsin, add medium that contain fetal bovine serum to terminate the digestion, thus to prepare the cell suspension. Centrifuge at 1000 g for 5~10 min and collect cells, then wash with serum-free medium for 1~2 times. Centrifuge and collect cell precipitation for fluorescence detection.

### 2. Fluorescence detection:

a. Re-suspend collected cells with serum-free medium for detection.

b. Wavelength: the excitation wavelength is 500 nm, the emission wavelength is 525 nm. It can also be detected according to the fluorescence detection conditions of FITC.

**Note:**

The density of re-suspension cell is determined by cell fluorescence intensity. If fluorescence is strong (weak), then decrease (increase) the cell density, but cell density of all samples should be consistent.

## ▲Detection of tissue sample

### 1. Preparation of single cell suspension:

Method 1: using the single cell suspension instrument.

Method 2: enzyme digestion.

a. Take the tissue into pre-cooled PBS（0.01 M，pH 7.4）immediately and clean the blood and other contaminants. Remove the massive composition, fiber, fat, and blood vessels (except for specialized cells).

b. Cut the tissue into about 1 $mm^3$ pieces with the ophthalmic scissors, then put these pieces to pre-cooled PBS（0.01 M，pH 7.4）to remove the cell debris.

c. Add an appropriate amount of enzyme digestion, incubate in 37℃ water bath for 20~30 min and gently oscillate the mixture intermittently.

d. Stop the digestion with medium that contain fetal bovine serum. Filter the mixture to remove the tissue massive component with nylon mesh (300 mesh) and collect the cells. Centrifuge at 500 g for 10 min and discard the supernatant, then wash with PBS（0.01 M，pH 7.4）for 1~2 times. Re-suspend to prepare the single cell suspension solution. The cell amount should be no less than $10^6$.

Method 3: mechanical method.

a. The pretreatment is the same as step a and step b in the enzyme digestion method.

b. Tight the nylon mesh (300 mesh) on a small beaker, then place the tissue pieces on the mesh and gently rub the tissue with ophthalmic scissor or erasing knife. Wash the tissue with PBS（0.01 M，pH 7.4）at the same time.

c. Collect the cell suspension and centrifuge at 500 g for 10 min. Then discard the supernatant and wash with PBS（0.01 M，pH 7.4）for 1~2 times. Re-suspend to prepare the single cell suspension solution. The cell amount should be no less than $10^6$.

### 2. Add the fluorescent probe:

a. Add Reagent 1 working solution to the cells. The DCFH-DA working concentration can be 0.1-20 μM for different cells and treatment. Pre-experiment is suggested to determine the appropriate concentration. The total dilution ratio should be more than 1:500-1:1000 in order to avoid effects of DMSO on cells. DMSO should be set as solution control.

b. Incubate at 37℃ for 30 min ~ few hours, generally 30~60 min. The incubation time is related to cell types, stimulation conditions, and DCFH-DA concentration.

c. Collect the incubated single cell suspension, centrifuge at 1000 g for 5~10 min to collect cells. Wash with serum-free medium for 1~2 times. Centrifuge and collect the cell precipitation for fluorescence detection.

### 3. Fluorescence detection:

a. Re-suspend collected cells with serum-free medium for detection.

b. Wavelength: the excitation wavelength is 500 nm, the emission wavelength is 525 nm. It can also be detected according to the fluorescence detection conditions of FITC.

## Notes

1. The density of re-suspension cell is determined by cell fluorescence intensity. If fluorescence is strong (weak), then decrease (increase) the cell density, but cell density of all samples should be consistent.
2. Fluorescent substances are sensitive to light and should avoid light during detection.
3. DCF is easy to be quenched, and the samples after incubation must be detected within 2 hours.
4. Results were expressed as fluorescence intensity or geometric average fluorescence intensity (flow cytometry).
5. When using flow cytometry, in order to avoid the interference of cell debris and dead cells on the experimental results, it is necessary to eliminate them.
6. Set a positive control (reagent 2 working solution) and a negative control (only cells without reagent 1 working solution).
7. The timing of adding DCFH-DA or incubation time depends on whether the intracellular reactive oxygen species can be detected successfully. DCFH-DA can be added in advance or at the same time if the drug treatment time is short (<2 h) or the predicted ROS is weak. Conversely, DCFH-DA can be added later if the drug treatment time is long (>6 h) or predicted ROS is strong.

# Appendix References


1. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction[J]. Annu Rev Plant Biol, 2004, 55: 373-399.
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# 过氧化氢检测试剂盒

| 产品编号 | 产品名称 | 包装 |
|---|---|---|
| S0038 | 过氧化氢检测试剂盒 | 150次 |

## 产品简介：

- 过氧化氢检测试剂盒(Hydrogen Peroxide Assay Kit)可以用于培养细胞或组织内过氧化氢水平的测定，也可以用于培养细胞的上清或血清、尿液、血浆或其它生物体液中的过氧化氢浓度的测定。
- 过氧化氢是一种活性氧代谢的副产物，在许多氧化应急反应中过氧化氢都是一种关键的调节因子。过氧化氢可以激活NF-κB等因子，这些过氧化氢相关的信号途径和哮喘、炎症性关节炎、动脉硬化以及神经退行性疾病等许多疾病相关。过氧化氢也和细胞凋亡、细胞增殖等密切相关。
- 本试剂盒通过过氧化氢氧化二价铁离子产生三价铁离子，然后和xylenol orange在特定的溶液中形成紫色的产物，从而实现对过氧化氢浓度的测定。本试剂盒经过改良配方，可以检测低达1微摩尔/升的过氧化氢。
- 本试剂盒方便快捷，通常10-20个样品可以在40-60分钟内测定完毕。
- 本试剂盒可以检测150个样品。

## 包装清单：

| 产品编号 | 产品名称 | 包装 |
|---|---|---|
| S0038-1 | 过氧化氢检测试剂 | 15ml |
| S0038-2 | 过氧化氢标准溶液(1M) | 1ml |
| S0038-3 | 过氧化氢检测裂解液 | 50ml |
| — | 说明书 | 1份 |

## 保存条件：

-20ºC保存，一年有效。其中过氧化氢标准溶液需避光保存。

## 注意事项：

- 一些干扰氧化还原的试剂或在酸性条件下呈紫色或接近的试剂会对过氧化氢的检测产生干扰，需尽量避免。
- 如果样品中含有外加的较高浓度的铁盐，会干扰测定。但普通培养基、血清等样品中含有的微量的铁盐不会干扰测定。
- 所测得的标准曲线尽管在一定的浓度范围内接近直线，但整个标准曲线不是直线。
- 测定时需可以测定A560的酶标仪一台(测540-570nm也可以)或可以测定微量样品的分光光度计一台。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

## 使用说明：

**1. 样品测定的准备：**

a. 细胞或组织样品的制备

对于培养的细胞，先收集细胞到离心管内，弃上清，按照每100万细胞加入100-200微升过氧化氢检测裂解液的比例加入裂解液，随后充分匀浆以破碎并裂解细胞。4ºC约12000g离心3-5分钟，取上清，用于后续测定。组织样品按照每5-10mg组织加入100-200微升裂解液的比例进行匀浆。4ºC约12000g离心3-5分钟，取上清用于后续测定。以上所有操作均需在4ºC或冰上操作。制备好的细胞或组织样品如果不立即测定，可以-20ºC冻存。

b. 培养细胞上清液样品的制备

培养细胞的上清液可以直接用于后续的测定。

c. 血清、血浆或尿液样品的准备：

配制50mM磷酸缓冲液，pH为6.0。用pH为6.0的50mM磷酸缓冲液把样品稀释50倍。例如4微升样品稀释到196微升pH为6.0的50mM磷酸缓冲液中。稀释后即可用于后续的测定。

**2. 标准曲线测定的准备：**

a. 过氧化氢标准品的校准：

由于过氧化氢不是非常稳定，使用前需自行测定过氧化氢的实际浓度以进行校准。把浓度约为1M的过氧化氢用水稀释100倍，使过氧化氢的浓度约为10mM，测定$A_{240}$。$A_{240}$的测定可采用如下的任一方法：

(a) **普通紫外分光光度计法：**使用含比色皿架的紫外分光光度计、NanoDrop 2000C、NanoDrop One$^c$、QuickDrop等仪器，配套石英比色皿。确定比色皿光程(path length)，一般为1cm。用比色皿检测的过氧化氢浓度最接近实际浓度。

(b) **微量紫外分光光度计法：**如NanoDrop 2000、NanoDrop One、QuickDrop、含超微量检测板μDrop Plate的Varioskan等仪器。确定光程：对于NanoDrop 2000、NanoDrop One等，需要取消“自动化光程”，此时光程一般为0.1cm；Varioskan的超微量检测板μDrop Plate的光程一般为0.05cm。具体的微量紫外分光光度计的光程请参考仪器参数。

(c) **96孔紫外酶标仪法(须能检测240nm波长)：**根据96孔板的参数确定光程，一般200微升样品的光程为0.552cm (样品体积除以96孔单孔孔内横截面面积)。一般建议使用专用的96孔紫外检测板(如96孔UV板)，如果没有紫外检测板，也可使用一般的96孔板，但由于为非紫外检测专用板，会有非常高的紫外吸收信号，所以需要设置含等量双蒸水的孔作为空白对照(一般200μl水在该类96孔板的$A_{240}$在3.8左右)，计算时须减去该空白对照。在使用非紫外检测专用板的情况下，由于96孔酶标仪在240nm的检测上限有限，建议将过氧化氢稀释至约10mM左右后再进行浓度测定。

**注意：以上所有方法都需要设置等量双蒸水作为空白对照，并在计算时减去该空白对照。**

浓度计算公式：c=A/(ε×b)。其中：c为样品浓度(单位为mol/L或M)；A为吸光值；ε为波长依赖的摩尔消光系数(单位为$L\times mol^{-1}\times cm^{-1}$或$M^{-1}\times cm^{-1}$)，过氧化氢的摩尔消光系数为$43.6M^{-1}cm^{-1}$；b=光程(单位为cm)。

因此：过氧化氢浓度(M)=$A_{240}$/(43.6×b)；即：过氧化氢浓度(mM)=22.94×$A_{240}$/b

从而计算出本试剂盒提供的过氧化氢的实际浓度，并根据实际测定出来的浓度进行后续的标准曲线的设置。

示例：将本试剂盒提供的约为1M的过氧化氢用双蒸水稀释100倍后，用96孔酶标仪及一般的96孔板进行检测，每孔200微升，每组3个平行。双蒸水对照组的平均$A_{240}$为3.750，过氧化氢样品组的平均$A_{240}$为3.974，差值为0.224，200微升样品的光程为0.552cm。代入公式，过氧化氢浓度(mM)=22.94×0.224/0.552=9.31，则实际本试剂盒提供的过氧化氢浓度为0.931M。

b. 标准曲线的设置：

样品在什么溶液中标准品也需用什么溶液稀释，这样可以减小误差。例如对于细胞样品，标准品宜用过氧化氢检测裂解液稀释，对于培养细胞的上清液样品，标准品宜用相应的细胞培养液稀释。标准溶液可以稀释成1、3、10、30、100微摩尔/升，或1、2、5、10、20、50、100微摩尔/升，初次测定后知道样品的浓度范围后可以对标准品在样品浓度范围附近密集测定。

**3. 过氧化氢浓度的测定：**

a. 把过氧化氢检测试剂在冰上或冰水浴上融解。
b. 在检测孔或检测管内加入50微升样品或标准品。
c. 在每个孔内加入100微升过氧化氢检测试剂。
d. 轻轻振荡或敲打混匀，室温(15-30ºC)放置30分钟。然后立即测定A560。如测A560有困难，波长可以选择540-570nm。
e. 根据标准曲线计算出样品中过氧化氢的浓度。

**注意：**如果样品中过氧化氢的浓度过高，可以适当稀释后再测定。如果样品中过氧化氢的浓度过低，可以把样品的体积改为使用100微升，同时标准品也使用100微升，而检测试剂仍然使用100微升。这样可以提高检测的灵敏度，但缺点是样品需要消耗100微升。

## 使用本产品的文献：


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Version 2020.03.20