

Supporting Information

Monoclonal antibody that recognizes diethoxyphospho-tyrosine modified proteins and peptides independent of surrounding amino acids

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Reagents for characterizing monoclonal antibody depY

Table S1 lists the diethoxyphosphorylated proteins prepared for this project. Appendix S1 identifies each modified residue and the ratio of labeled to unlabeled amino acids.

Table S1. Proteins diethoxyphosphorylated by chlorpyrifos oxon (CPO).

Protein name	Accession number
Human albumin	P02768
Mouse albumin	P07724
Aprotinin; pancreatic trypsin inhibitor, bovine	P00974
Casein bovine isozyme mixture	
Alpha S1	P02662
Alpha S2	P02663
Beta	P02666
CSN2	P02666
kappa	P02668
Lysozyme gallus gallus	P00698
Bovine tubulin	
Alpha1B	P81947
Beta-7	E1B953
Beta-4B	Q3MHM5
Porcine tubulin	
Alpha-1A	P02550
Alpha-1B	Q2XVP4
Beta	Q767L7
Beta	P02554

Casein is the most informative OP-modified protein for our purposes because tyrosines were exclusively modified by reaction with CPO. In contrast, human albumin, mouse albumin, bovine tubulin, and porcine tubulin were modified on tyrosines and lysines.

Table S2 lists diethoxyphosphate-tyrosine labeled peptides conjugated to carrier proteins. Mass spectrometry identified OP-labeled tyrosine as the modified residue in each peptide before it was crosslinked to the carrier protein. The number of OP-peptides per protein molecule was determined by MALDI-TOF mass spectrometry as illustrated in Figure S1.

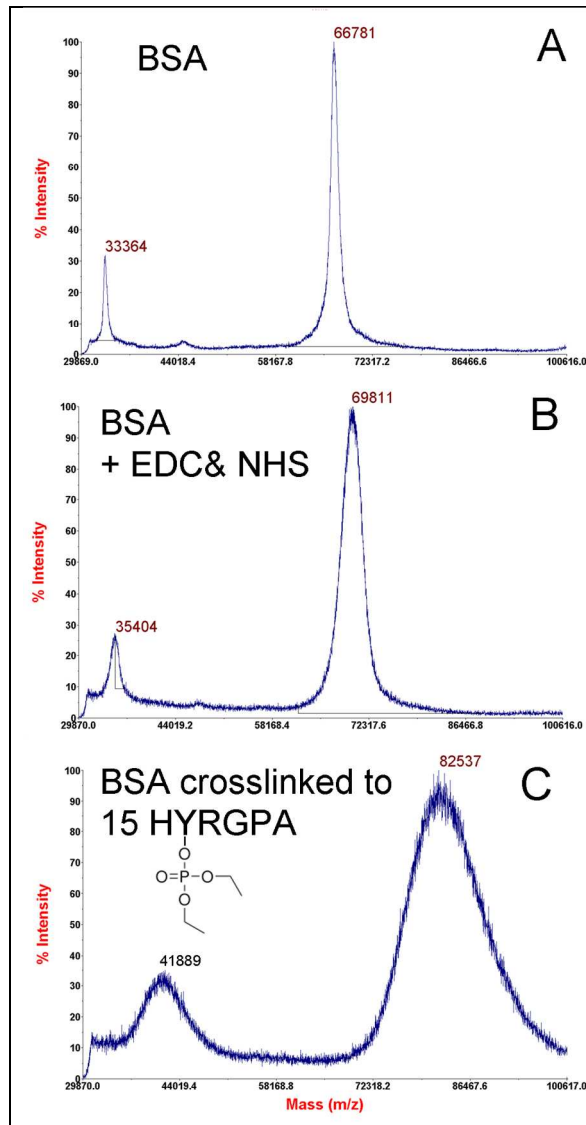


Figure S1. Quantitation of number of OP-peptides bound per molecule of bovine albumin (BSA). Maldi-TOF spectra were acquired in linear mode for samples in sinapinic acid.

A) peaks for doubly and singly charged BSA.

B) BSA in the presence of the crosslinking agents EDC and sulfoNHS.

C) BSA crosslinked to HYRGPA-OP modified on tyrosine by diethoxy phosphate. The difference in mass between 82537 (panel C) and 69811 (panel B) is 12726, which divided by 835 for the mass of the OP-peptide, calculates to 15 peptides bound per molecule of BSA.

Table S2. Diethoxyphospho-tyrosine peptides conjugated to carrier proteins.

For immunization; mixture of bovine albumin (BSA) conjugated to 5 different OP-peptides, in 0.1 M MES pH 6.2

BSA + LYGLPR-OP; 16 peptides/BSA	1 mg in 400 μ L
BSA + KYA-CPO; 19 peptides/BSA)	1 mg in 400 μ L
BSA + PVYSR-OP; 16 peptides/BSA	1 mg in 400 μ L
BSA + HYRGPA-OP; 15 peptides/BSA	1 mg in 400 μ L
BSA + EPNVSY-OP; 10 peptides/BSA	1 mg in 400 μ L

For boosting; mixture of ovalbumin (OVA) and human albumin (HSA) conjugated to 5 different OP-peptides

OVA + RSLYAS-OP; 18 & 22 peptides/OVA	1 mg in 400 μ L
OVA + YGGFL-OP; 9, 5 & 12 peptides/OVA	1 mg in 400 μ L
OVA + YPF-OP; 10, 6 & 14 peptides/OVA	1 mg in 400 μ L
HSA + PPYRM-OP; 22 peptides/HSA	1 mg in 400 μ L
HSA + QYDVRK-OP; 7 peptides/HSA	1 mg in 400 μ L

For screening hybridoma culture media; mixture of lysozyme conjugated to 3 different OP peptides

Lysozyme + RARYEM-OP; 6 & 9 peptides/lysozyme	1 mg in 400 μ L
Lysozyme + GGYR-OP; 4, 10 & 16 peptides/lysozyme	2 mg in 800 μ L
Lysozyme + KYK-OP; 1, 5, 9 & 12 peptides/lysozyme	2 mg in 800 μ L

For re-screening hybridoma culture media

Human albumin-OP	in Appendix 1
Mouse albumin-OP	in Appendix 1

For ELISA and Western blots
 HSA + YGGFL-OP; 5 peptides/HSA
 HSA + QYDVRK-OP; 7 peptides/HSA
 Lysozyme-PYYMRR-OP
 BSA-LYGLPR-OP; 16 peptides/BSA
 OVA-RSLYAS-OP; 18 & 22 peptides/OVA

Table S3. Diethoxyphosphate-tyrosine peptides (no protein)

RARYEM-CPO	HYRGPA-CPO
GGYR-CPO	PPYRM-CPO
GGYR-paraoxon	QYDVRK-CPO
KYK-CPO	VSYSK-CPO
RSLYAS-CPO	LYGLPR-paraoxon
GTYKW-paraoxon	SGYTR-CPO
PYYMRR-CPO	YGGFL-CPO

Table S4. Additional reagents for characterizing the specificity of depY

Human albumin-lysine-OP 14/albumin
 Human albumin-phospho-tyrosine 4/albumin
 Human BChE modified on serine 198 with CPO
 Human BChE modified on serine 198 with phosphate
 Human BChE modified on serine 198 with cresylphosphate
 Human BChE modified on serine 198 with dichlorvos

Appendix S1. Identification and quantitation of residues modified by reaction of proteins with chlorpyrifos oxon.

Procedure. 1 mg/ml protein (human albumin, bovine tubulin, porcine tubulin, casein, lysozyme, aprotinin) in 20 mM TrisCl pH 8.9, 0.01% sodium azide was incubated at 37°C with chlorpyrifos oxon (Chem Service, MET 674B) dissolved in ethanol to make 1.5 mM CPO. At the end of the 7-day reaction period, disulfide bonds were reduced and alkylated. Samples were dialyzed against 10 mM ammonium bicarbonate, 0.01% azide at 4°C, before they were digested with pepsin or trypsin. Peptic peptides were analyzed on the 4800 MALDI-TOF/TOF mass spectrometer, where % labeled was calculated from relative peak heights. Tryptic peptides were analyzed by LC-MS/MS on the 6600 Triple TOF mass spectrometer where % labeled was calculated from spectral counts.

Human albumin P02768

1 mkwvtfisll flfssaysrg vfrrdahkse vahrfkdlge enfkalvlia faqylqqcpcf
 61 edhvklnvnev tefaktcvad esaencdksl htlfgdklct vatlretyge madccakqep
 121 ernecflqh^k ddnpnlprlv rpevdvmcta fhdneetflk k^vlyeiarrh pyf^vapellf
 181 fakry^kaaft eccqaadkaa cllpkldelr deg^kassakq rl^kcaslqkf geraf^kawav
 241 arlsqrfpka efaevsklvt dltkvhtecc hgdlllecadd radlakyice nqdsissklk
 301 eccekpillek shciaevend empadlpsla adfveskdvc knyaeakdvf lgmflyeyar
 361 rhpdyssvll lrlaktyett lekccaaadp hecyakvfde fkplveepqn likqncelfe
 421 qlge^vkfqna llvr^vtk^kvp qvstptlvev srnlq^kvgsk cckhpeakrm pcaedylsv
 481 lnqlcvlhek tpsvdrvtkc cteslvnrrp cfsalevdet yvpkefnaet ftfhadictl
 541 sekerqikkq talvelvkhk pkat^keqlka vmddfaafve kckkaddket cfaeegkklv
 601 aasqaalg1

Residue #	% labeled with diethoxyPhosphate (+136)		% labeled with diethoxyPhosphate (+136)
Y162 (138)	21%	K130 (106)	14%
Y174 (150)	2%	K186 (162)	16%
Y425 (401)	5%	K214 (190)	67%
Y435 (411)	98%	K223 (199)	85%
		K236 (212)	40%
		K438 (414)	4%
		K455 (431)	100%

		K565 (541)	33%
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Numbers in parentheses are residue numbers minus the 24 amino acid signal peptide. Chlorpyrifos made a covalent bond with 4 tyrosines and 8 lysines. This preparation of human albumin-OP was used in ELISA to identify 4 positives in the screen of 30 hybridoma culture media received from SydLabs. It is negative on Western blots hybridized with depY but positive in ELISA.

Mouse albumin P07724

1 mkwvtflllll fvsqgsafsrq vfrreahkse iahryndlge qhfkglvlia fsqylqkcsy
61 dehaklvqev tdfaktcvad esaancdksl htlfqgdklca ipnlrenyge ladcctkqep
121 ernecflqhk ddnpplppfe rpeaeamcts fkenpttfmg hylhevarrh pyfyapelly
181 yaeqyneilt qccaeadkes cltpkldgvk ekalvssvrq rmkcssmqkf gerafkawaw
241 arlsqtfpna dfaeitklat dltkvnkecc hgdllecadd raelakymce nqatissklq
301 tccdkpllkk ahclsevehd tmpadlpaia adfvedqevc knyaeakdvf lgtflyeysr
361 rhpdyvs1l lrlakkyeat lekccaeapn pacygtvlae fqplveepkn lvktncdlye
421 klgeygfqna ilvrvtqkap qvstptlvea arnlgrvgtk cctlpedqrl pcedyylsai
481 lnrvc1lhek tpvsehvtkc csgslverrp cfsaltvdet yvpkefkaet ftfhsdictl
541 pekekqikkq talaelvkhk pkataeqlkt vmddfaqfld tcckaadkdt cfstegpnlv
601 trckdala

Residue #	% labeled with diethoxyPhosphate (+136)	Residue #	% labeled with diethoxyPhosphate (+136)
Y435 (411)	83	K210 (186)	85
		K212 (188)	14
		K223 (199)	100
		K236 (212)	40
		K376 (352)	11
		K460 (436)	43

Numbers in parentheses are residue numbers minus the 24 amino acid signal peptide. Chlorpyrifos made a covalent bond with 1 tyrosine and 6 lysines. This mouse albumin-OP preparation was used for screening 30 hybridoma culture media.

Aprotinin, Pancreatic Trypsin Inhibitor. Bos Taurus (bovine) P00974

1 msrlslktsg deenwvsrfr skslslvfsg vlalglslsg vgfadarpdf cleppvtgpc
61 kariiryfyn akaglcqtfv yggcrakrnn fksaedcmrt cgga

Residue #	% labeled with diethoxyPhosphate(+136)	Residue #	% labeled with diethoxyPhosphate(+136)
Y56	10	K92	65

This preparation gave a weak positive signal on Western blot hybridized with depY.

Casein from bovine milk Sigma C5890 is a mixture of isoforms

Alpha S1 46% 8-10 phosphates per mole
Alpha S2 12% 10-13 phosphates per mole
Beta 35% 4-5 phosphates per mole
Kappa 8% 1 phosphate per mole

Casein alpha S1 Bos Taurus P02662

1 MKLLILTCLV AVALARPKHP IKHQGLPQEV LLENLLRFFV APFPEVFGKE KVNELSKDIG
61 SESTEDQAME DIKQMEAESI SSSEQKHIQK EDVPSERYLG YLEQLLRLKK YKVPQLEIVP
121 NQAEERLHSM KEGIHAQQKE PMIGVNQELA YFYPELFRQF YQLDAYPSGA WYYVPLGTQY
181 TDAPSFSDIP NPIGSENSEK TTMLPW

Residue #	% labeled diethoxyPhosphate (+136)	Residue #	% labeled phosphate (+80)
Y111	40	S122	91
Y151	9		
Y153	9		

Phosphosite shows 9 phosphorylated serine residues in casein alpha S1, of which only one was detected by the 6600 Triple TOF mass spectrometer.

Casein alpha S2 Bos Taurus P02663

1 MKFFIFTCLL AVALAKNTME HVSSSEESII SQETYKQEK N MAINPSKENL CSTFCKEVVR
 61 NANEEEEYSIG SSSEESA EVA TEEVKITVDD KH^YQKALNEI NQF^YQKFPQY LQYLYQGPIV
 121 LNPWDQVQRN AVPIPTLNR EQLSTSEENS KKTVDMESTE VFTKTKL TE EEKNRLNFLK
 181 KISQR^YQKFA LPQYLKTVYQ HQKAMKPW IQ PKTKVIPYVR YL

Residue	% labeled +136)
Y93	5
Y104	17
Y186	95

Phosphosite shows 18 phosphorylated serine and threonine in casein alpha S2.

Casein beta (CSN2 protein) Bos Taurus P02666

1 MKVLILACL V ALALARELEE LNVPG EIVES LSSSEESITR INKKIEKFQ^S EEQQQTEDEL
 61 QDKIH PFAQT QSLVY PFGP IPNSLPQNIP PLTQTPVVVP PFLQPEVLGV SKVKEAMAPK
 121 HKEMPF PKYP VEPFTE SQSL TLTDVENLHL PLLLLQSWMH QPHQPLPPTV MFPPQSVL SL
 181 SQSKVLPV PQ KAVP^YPQRDM PIQAFLLYQ Q PVLGPVRGPF PIIV

Residue #	% labeled diethoxyPhosphate (+136)	Residue #	% labeled phosphate (+80)
Y195	10	S50	87

Phosphosite shows 7 phosphorylated serine and threonine residues in casein beta.

Casein CSN2 Bos Taurus P02666

1 MKVLILACL V ALALARELEE LNVPG EIVES LSSSEESITR INKKIEKFQ^S EEQQQTEDEL
 61 QDKIH PFAQT QSLVY PFGP IHNSLPQNIP PLTQTPVVVP PFLQPEVMGV SKVKEAMAPK
 121 HKEMPF PK^Y VEPFTE SQSL TLTDVENLHL PLPLLQSWMH QPHQPLPPTV MFPPQSVL SL
 181 SQSKVLPV PQ KAVP^YPQRDM PIQAFLLYQ Q PVLGPVRGPF PIIV

Residue #	% labeled diethoxyPhosphate (+136)	Residue #	% labeled phosphate (+80)
Y129	26	S50	89
Y195	10		

Casein kappa Bos Taurus P02668

1 MMKSFFLVVT ILALTL PFLG AQEQNQEQPI RCEKDERFFS DKIAKYIPIQ ^YVLSRYP SYG
 61 LNY YQK PVA LINNQFLPYP YYAKPA AVRS PAQILQWQVL SNTVPAKSCQ AQPTT MARHP
 121 HPHLSFMAIP PKNQDKTEI PTINTIASGE PTSTPTTEAV ESTVATLEDS PEVIESPPEI
 181 NTVQVTSTAV

Residue	% labeled (+136)
Y51	50

Phosphosite shows 4 phosphorylated serine and threonine in casein kappa.

This casein-OP preparation gave a strong positive signal in Western blots and ELISA hybridized with depY.

Lysozyme Gallus gallus P00698

Red residues are labeled with diethoxyphosphate.

Blue residues are labeled with phosphate.

Green residues are labeled with both diethoxyphosphate and phosphate.

1 KVFGRC ELAA AM^KRHGLDN^Y RGY^SLGDWVC AA^KFESNFNT QATNRNTDG^S T^D^YGILQINS
 61 RWWCNDGRTP GSRNLCNIPC ^SALL^{SS}DITA ^SVNCA^{KK}IVS DGNGMNAWVA WRNRC^KGTDV
 121 QAWIRGCR L

Residue #	% labeled diethoxyPhosphate (+136)	% labeled phosphate (+80)
Y20	low	
Y23	3	1
Y53	5	
S24	low	Low
K13	4	
K33	7	
S50	Low	1
T51	Low	2

S81	Low	1
S85	Low	Low
S86	Low	low
S91		low
K96	9	Low
K97	1	low
K116	3	3

Low means less than 1% labeled. Phospho.elm.eu.org shows two serines (S24 and S50) phosphorylated in lysozyme C (egg white) P00698 gallus gallus.

Lysozyme modifications are sparse and unspecific; the OP adduct is on lysines, tyrosines, serines and threonine. Lysozyme-OP was negative in Western blot, but weakly positive in ELISA with monoclonal antibody depY.

tubulin alpha-1B chain [Bos taurus] P81947

1 mrecisihvg qagvqignac welyclehgi qpdgqmpsdk tigggddsfn tffsetgagk
61 hvpravfdvl eptvidevrt gtyrqlfhpe qlitgkeda nnyarghyti gkeiidlvld
121 rirkkladqct glqgflvfhs fgggtgsgft sllmerlsvd ygkksklefs iypapqvsta
181 vvepynsilt thttlehsdc afmvdneaiy dicrrndie rptytnlnrl isqivssita
241 slrfdgalnv dltefqtlnv p[]prihfpla tyapvisaek ayheqlsvae itnacfeapan
301 qmvkcdprhg kymaccllyr gdvvpkdvna aiatiktksr iqfvdwceptg fkvginyqpp
361 tvvpggdlak vqravcmlsn ttaiaeawar ldhkfdlmya krafvhwvyg egmeegefse
421 aredmaalek dyeevgvdsv egegeeegee y

Residue	% labeled diethoxyPhosphate (+136)
Y262	12 %

1 tyrosine modified, 18 not modified

PREDICTED: tubulin beta-7 chain [Bos taurus] E1B953

NCBI Reference Sequence: XP_612678.3

1 mreivhiqag qcgnqigakf wevisdehgi datgtyhgds dlqldrisv[] [input type="checkbox"/>]neatggkyv
61 prailvdlep gtmdsvhsgp fgqifrsdni vfgqsgadnn wakghyrega elvdsvladv
121 rkeakscdcl qgfqlthslg ggtsgmgmtl liskireeyp dhimntfsv pspkvsdtvv
181 epynatlsvh qlventdety cidnealyai cftrtlklttp tygdlnqlvs atmsgvttcl
241 rfpqqlnadl hklavnmvpf phlhffvpgf apltscgsqq ywaltvpelt qqvfdaknmm
301 aacdprhgr[] ltvaavfrgq msmkevdeqm lnvqknss[] fvewipnnvk tavcdipprg
361 lkmavtfign staiqelfkr iseqlmamfr r[]aflhwytg egmdetefte aesnmndlvs
421 eyqqyqdata eeedfgeea eeee

Residue	% labeled diethoxyPhosphate (+136)
Y50	26%
Y51	14%
Y310	23%
Y340	19%
K392	71%

4 tyrosines modified, 12 tyrosines not modified; 1 lysine modified, 15 lysines not modified

tubulin beta-4B chain [Bos taurus] Q3MHM5

1 mreivhlqag qcgnqigakf wevisdehgi dptgtyhgds dlqlerinv[] [input type="checkbox"/>]neatggk[]v
61 pravlvdllep gtmdsvrsgp fgqifrpdnf vfgqsgagnn wakgh[]tega elvdsvladv
121 rkeaescdcl qgfqlthslg ggtsgmgmtl liskireeyp drimntfsv pspkvsdtvv
181 epynatlsvh qlventdety cidneal[]di cftrtlklttp tygdlnhlvs atmsgvttcl
241 rfpqqlnadl rklavnmvpf prlhffmpgf apltsrgsqq yraltvpelt qqmfdaknmm
301 aacdprhgr[] ltvaavfrgr msmkevdeqm lnvqknss[] fvewipnnvk tavcdipprg

361 lkmsatfign staiqelfkr iseqftamfr r[**k**]aflhwytg egmdemefte aesnmndlvs
 421 eyggygdata eeegefefeeea eeeva

Residue	% labeled diethoxyPhosphate (+136)
Y50	18
Y51	15
Y59	21
Y106	4
Y208	26
Y310	23
Y340	19
K392	71

7 tyrosines modified, 9 tyrosines not modified; 1 lysine modified, 14 lysines not modified

Tubulin alpha-1A chain OS=Sus scrofa P02550

1 mrecisihvg qagvqignac welyclehgi qpdgqmpsdk tigggddsfn tffsetgagk
 61 hvpravfvdI eptvidevrt gt[**v**]rqlfhpe qlitg[**k**]edaa nn[**v**]arghyti g[**k**]eiidlvld
 121 rirkIadqct glqgfsvfhs fgggtgsgft sllmerIsvd [**v**]g[**k**]s[**k**]lefs i[**v**]papqvsta
 181 vvepynsilt thttlehsdc afmvdneaiy dicrrnldie rpt[**v**]tnlnrl igqivssita
 241 slrfdgalnv dltefqtnlv p[**v**]prahfpla t[**v**]apvisaek a[**v**]heqlsvae itnacfePan
 301 qmvkcdprhg [**k**]ymaccllyr gdvvp[**k**]dvna aiati[**k**]t[**k**]rt iqfvdwcptg fkvgin[**v**]epp
 361 tvvpggdla[**k**] vqravcmlsn ttaiaeawar ldh[**k**]fdlm[**v**]a [**k**]rafvhw[**v**]vg egmeegefse
 421 aredmaalek dyeevgvdsv egegeeegee y

Residue #	% Labeled (+136)	Residue #	% Labeled (+136)
Y83	9	K96	16
Y103	69	K112	21
Y108	42	K163	27
Y161	43	K164	24
Y172	14	K166	17
Y224	19	K311	55
Y262	16	K326	41
Y272	37	K336	11
Y282	10	K338	8
Y312	45	K370	30
Y357	7	K394	33
Y399	16	K401	38
Y408	18		

Tubulin alpha-1B chain OS=Sus scrofa Q2XVP4

1 mrecisihvg qagvqignac welyclehgi qpdgqmpsdk tigggddsfn tffsetgagk
 61 hvpravfvdI eptvidevrt gt[**v**]rqlfhpe qlitg[**k**]edaa nn[**v**]argh[**v**]ti g[**k**]eiidlvld
 121 rirkIadqct glqgflvfhs fgggtgsgft sllmerIsvd [**v**]g[**k**]s[**k**]lefs i[**v**]papqvsta
 181 vvepynsilt thttlehsdc afmvdneaiy dicrrnldie rpt[**v**]tnlnrl isqivssita
 241 slrfdgalnv dltefqtnlv p[**v**]prihfpla t[**v**]apvisaek a[**v**]heqlsvae itnacfePan
 301 qmvkcdprhg [**k**]ymaccllyr gdvvp[**k**]dvna aiati[**k**]t[**k**]rs iqfvdwcptg f[**k**]vgin[**v**]qpp
 361 tvvpggdla[**k**] vqravcmlsn ttaiaeawar ldh[**k**]fdlm[**v**]a [**k**]rafvhw[**v**]vg egmeegefse
 421 aredmaalek dyeevgvdsv egegeeegee y

Residue #	% Labeled (+136)	Residue #	% Labeled (+136)
Y83	6	K96	16
Y103	67	K112	25
Y108	17	K163	30
Y161	28	K164	14
Y172	25	K311	55

Y224	27	K326	8
Y262	27	K336	4
Y272	31	K338	6
Y282	27	K352	71
Y312	45	K370	19
Y357	7	K394	48
Y399	33	K401	60
Y408	18		

Tubulin beta chain OS=Sus scrofa Q767L7

1 mreivhiqag qcgngigakf wevisdehgi dptgtyhgds dlqldrisvy yneatggk[**V**]
61 prailvdlep gtmdsvrsgp fgqifrpdnf vfgqsgagnn wak[**gh**][**V**]tega elvdsvldvv
121 rkeaescdcl qgfqlthslg ggtgsgmgtl lis[**k**][**i**][**r**][**e**][**e**][**v**][**p**]drimntfsvv pspkvsdtvv
181 ep[**v**]natlsvh qlventdety cidnealydi cfrtl[**k**]lttp tygdlnhlvs atmvgvttcl
241 rfpqqlnadl r[**k**]lavnmpvf prlhffmpgf apltsrgsqg [**v**]raltvpelt qqvfdaknmm
301 aacdprhgr[**v**] ltvaavfrgr msmk[**e**]vdeqm lnvqknss[**v**] fvewipnnvk tavcdipprg
361 lkmavtfign staiqelf[**k**]r iseqftamfr r[**k**]afllhw[**v**]tg egmdemefte aesnmndlvs
421 eyqqyqdata eeedfgeea eeea

Residue #	% Labeled (+136)	Residue #	% Labeled (+136)
Y59	8	K103	24
Y106	12	K154	23
Y159	27	K216	50
Y183	7	K252	14
Y281	22	K324	15
Y310	35	K379	11
Y340	46	K392	100
Y398	36		

Tubulin beta chain OS=Sus scrofa P02554

1 mreivhiqag qcgngigakf wevisdehgi dptgsyhgds dlqlerinv[**v**] [v]neaagn[**ky**][**v**]
61 prailvdlep gtmdsvrsgp fgqifrpdnf vfgqsgagnn wak[**gh**][**v**]tega elvdsvldvv
121 rkesescdcl qgfqlthslg ggtgsgmgtl lis[**k**][**i**][**r**][**e**][**e**][**v**][**p**]drimntfsvv pspkvsdtvv
181 ep[**v**]natlsvh qlventdety cidnealydi cfr[**t**][**l**][**k**]lttp t[**v**]gdlnhlvs atmvgvttcl
241 rfpqqlnadl r[**k**]lavnmpvf prlhffmpgf apltsrgsqg [v]raltvpelt qqmfdaknmm
301 aacdprhgr[**v**] ltvaavfrgr msm[**k**]evdeqm lnvqknss[**v**] fvewipnnvk tavcdipprg
361 l[**k**]msatfign staiqelf[**k**]r iseqftamfr r[**k**]afllhw[**v**]tg egmdemefte aesnmndlvs
421 eyqqyqdata deggefefeeg eeeda

Residue #	% Labeled (+136)	Residue #	% Labeled (+136)
Y50	15	K58	18
Y51	12	K103	24
Y59	8	K154	16
Y106	12	K216	50
Y159	27	K252	42
Y183	7	K324	18
Y222	4	K362	47
Y281	17	K379	63
Y310	35	K392	100
Y340	30	T214	50
Y398	36		

Bovine and porcine tubulin-OP gave strong positive signals on Western blots and ELISA with monoclonal antibody depY.