

Phosphotyrosine Detection Kit

Order No.: 0703/PTYR-KIT



www.nanotools.de

orders & support:

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 fax: +49-7641-455 671

02/080507

Background and Specificity

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein interactions.

Modification of proteins on tyrosine residues is mediated by protein tyrosin kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via the interaction of phosphotyrosine residues with SH2 or PTB domains.

Antibodies direct against phosphorylated epitopes recognize the phosphorylated amino acid in the context of the surrounding amino acid sequence. Recognition is therefore dependent on 2 criteria: 1) phosphorylation and 2) the surrounding amino acid motif. If one of the two criteria is not fulfilled, the antibody will not detect the phosphorylation site. Since the amino acid sequence varies between different phosphorylation sites, certain proteins - though phosphorylated - may not be detected by the antibody. Phosphorylation patterns in a given cell extract may differ when probed with different antibodies due to sequence specificity.

The Phosphotyrosine Detection Kit contains 7 different phosphotyrosine specific monoclonal antibodies.

clone	isotype	order number
1F9	IgG1	0226-025
2A5	IgG1	0133-025
2C8	IgG1	0027-025
3B12	IgG1	0028-025
9F1	IgG1	0156-025
9H8	IgG1	0029-025
16F4	IgG1	0030-025

Postive control

This product contains the following positive control for immunoblot applications:

#0038S Phosphotyrosine Molecular Weight Marker

Mouse Monoclonal Antibody to

Phosphotyrosine

clone 1F9

Order No.: 0226-025/PTYR-1F9

Size (µg) 25

Lot No.: 0226S



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03/230807F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP	pattern		... P - E - pY - H - N ...	phosphopeptide conjugated to hemocyanin

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-1F9 recognizes phosphotyrosine in the context of the surrounding amino acids, tolerating charged amino acids directly neighboured to phosphotyrosine.

Related Products

mab against Phosphotyrosine

#0027-100/pTyr-2C8

#0028-100/pTyr-3B12

#0029-100/pTyr-9H8

#0030-100/pTyr-16F4

#0133-100/pTyr-2A5

#0156-100/pTyr-9F1

Purification:	The antibody was purified from serum-free cell culture supernatant by subsequent ultrafiltration and size exclusion chromatography.
Formulation:	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
Reconstitution:	Reconstitute with 1 ml H ₂ O (15 min, RT).
Stability:	For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.
	Avoid repeated freeze / thaw cycles.
Positive Control:	#0038: phosphotyrosine MW standard
Immunoblotting:	0.5 µg/ml for HRPO/ECL detection Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
Immunoprecipitation:	use at 1 - 10 µg per 10 ⁶ pervanadate-treated A431 cells
Immunocytochemistry:	ND
ELISA:	use at 0.05 µg/ml

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Mouse Monoclonal Antibody to

Phosphotyrosine

clone 2A5

Order No.: 0133-025/PTYR-2A5

Size (µg) 25

Lot No.: 0133S



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02/060307F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP	pattern		... E - pY - M ...	phosphopeptide conjugated to KLH

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-2A5 recognizes a broad spectrum of tyrosine phosphorylated proteins in crude cell extracts. mab 2A5 tolerates a negative charge N - terminal to the phosphotyrosine residue.

Related Products

mab against Phosphotyrosine

#0027-100/pTyr-2C8

#0028-100/pTyr-3B12

#0029-100/pTyr-9H8

#0030-100/pTyr-16F4

#0156-100/pTyr-9F1

#0226-100/pTyr-1F9

Purification:	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
Formulation:	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
Reconstitution:	Reconstitute with 1 ml H ₂ O (15 min, RT).
Stability:	For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.
	Avoid repeated freeze / thaw cycles.
Positive Control:	#0038: phosphotyrosine MW standard
Immunoblotting:	0.5 µg/ml for HRPO/ECL detection Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
Immunoprecipitation:	use at 1 - 10 µg per 10 ⁶ pervanadate-treated A431 cells
Immunocytochemistry:	ND
ELISA:	use at 0.1 µg/ml

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Mouse Monoclonal Antibody to

Phosphotyrosine

clone 2C8

Order No.: 0027-025/PTYR-2C8

Size (µg) 25

Lot No.: 0027S



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02/060307F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP	pattern		... G - pY - Y ...	phosphopeptide conjugated to KLH

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-2C8 recognizes a broad range of tyrosine--phosphorylated proteins in crude cell extracts and may therefore be particularly well-suited for the detection/screening of tyrosine phosphorylated proteins.

Related Products

mab against Phosphotyrosine

#0028-100/pTyr-3B12

#0029-100/pTyr-9H8

#0030-100/pTyr-16F4

#0133-100/pTyr-2A5

#0156-100/pTyr-9F1

#0226-100/pTyr-1F9

Purification:	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
Formulation:	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
Reconstitution:	Reconstitute with 1 ml H ₂ O (15 min, RT).
Stability:	For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.
	Avoid repeated freeze / thaw cycles.
Positive Control:	#0038: phosphotyrosine MW standard
Immunoblotting:	0.5 µg/ml for HRPO/ECL detection Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
Immunoprecipitation:	use at 1 - 10 µg per 10 ⁶ pervanadate-treated A431 cells
Immunocytochemistry:	ND
ELISA:	use at 0.1 µg/ml

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Mouse Monoclonal Antibody to

Phosphotyrosine

clone 3B12

Order No.: 0028-025/PTYR-3B12

Size (µg) 25

Lot No.: 0028S

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02/060307F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP, ICC	pattern			phosphotyrosine conjugated to KLH

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-3B12 recognizes phosphotyrosine in the context of the surrounding amino acids.

Related Products

mab against Phosphotyrosine

#0027-100/pTyr-2C8

#0029-100/pTyr-9H8

#0030-100/pTyr-16F4

#0133-100/pTyr-2A5

#0156-100/pTyr-9F1

#0226-100/pTyr-1F9

Purification:	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
Formulation:	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
Reconstitution:	Reconstitute with 1 ml H ₂ O (15 min, RT).
Stability:	For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.
	Avoid repeated freeze / thaw cycles.
Positive Control:	#0038: phosphotyrosine MW standard
Immunoblotting:	0.5 µg/ml for HRPO/ECL detection Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
Immunoprecipitation:	use at 1 - 10 µg per 10 ⁶ pervanadate-treated A431 cells
Immunocytochemistry:	use at 1 - 10 µg/ml.
ELISA:	use at 0.05 µg/ml

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Mouse Monoclonal Antibody to

Phosphotyrosine

clone 9F1

Order No.: 0156-025/PTYR-9F1

Size (µg) 25

Lot No.: 0156S



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02/060307F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP	pattern		... pY pY ...	phosphopeptide conjugated to KLH

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-9F1 recognizes phosphotyrosine in the context of the surrounding amino acids, tolerating hydrophobic amino acids and phosphotyrosine directly neighbored to phosphotyrosine.

Related Products

mab against Phosphotyrosine

#0027-100/pTyr-2C8

#0028-100/pTyr-3B12

#0029-100/pTyr-9H8

#0030-100/pTyr-16F4

#0133-100/pTyr-2A5

#0226-100/pTyr-1F9

Purification:	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
Formulation:	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
Reconstitution:	Reconstitute with 1 ml H ₂ O (15 min, RT).
Stability:	For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.
	Avoid repeated freeze / thaw cycles.
Positive Control:	#0038: phosphotyrosine MW standard
Immunoblotting:	0.5 µg/ml for HRPO/ECL detection Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
Immunoprecipitation:	use at 1 - 10 µg per 10 ⁶ pervanadate-treated A431 cells
Immunocytochemistry:	ND
ELISA:	use at 0.05 µg/ml

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Mouse Monoclonal Antibody to

Phosphotyrosine

clone 9H8

Order No.: 0029-025/PTYR-9H8

Size (µg) 25

Lot No.: 0029S



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02/060307F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP	pattern		... R - G - pY - V - P ...	phosphopeptide conjugated to KLH

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-9H8 recognizes phosphotyrosine in the context of the surrounding amino acids, tolerating positively charged amino acids N-terminal to phosphotyrosine.

Related Products

mab against Phosphotyrosine

#0027-100/pTyr-2C8

#0028-100/pTyr-3B12

#0030-100/pTyr-16F4

#0133-100/pTyr-2A5

#0156-100/pTyr-9F1

#0226-100/pTyr-1F9

Purification: The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.

Formulation: lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.

Reconstitution: Reconstitute with 1 ml H₂O (15 min, RT).

Stability: For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.

Avoid repeated freeze / thaw cycles.

Positive Control: #0038: phosphotyrosine MW standard

Immunoblotting: 0.5 µg/ml for HRPO/ECL detection
Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.

Immunoprecipitation: use at 1 - 10 µg per 10⁶ pervanadate-treated A431 cells

Immunocytochemistry: ND

ELISA: use at 0.05 µg/ml

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Mouse Monoclonal Antibody to

Phosphotyrosine

clone 16F4

Order No.: 0030-025/PTYR-16F4

Size (µg) 25

Lot No.: 0030S



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02/060307F

Isotype	Species Reactivity	Applications	Mol. Weight	Ref.Cell Line	Epitope	Immunogen
IgG1	human, mouse, rat, dog	WB, ELISA, IP	pattern		... D - I - pY - A - E ...	phosphopeptide conjugated to KLH

Background and Specificity:

Phosphorylation and dephosphorylation of cellular proteins are central steps in transducing extracellular signals to the cell nucleus. Phosphorylated epitopes may serve as docking sites for the assembly of protein complexes or may alter the 3-dimensional protein structure thus modulating enzymatic activity or the ability to undergo protein-protein-interactions. Modification of proteins on tyrosine residues is mediated by protein tyrosine kinases. Tyrosine phosphorylation may alter the biological activity or mediate the assembly of protein complexes via interaction of phosphotyrosine residues with SH2 or PID domains.

Mab PTYR-16F4 recognizes phosphotyrosine in the context of the surrounding amino acids, tolerating hydrophobic amino acids directly neighboured to phosphotyrosine.

Related Products

mab against Phosphotyrosine

#0027-100/pTyr-2C8

#0028-100/pTyr-3B12

#0029-100/pTyr-9H8

#0133-100/pTyr-2A5

#0156-100/pTyr-9F1

#0226-100/pTyr-1F9

Purification: The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.

Formulation: lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.

Reconstitution: Reconstitute with 1 ml H₂O (15 min, RT).

Stability: For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months.

Avoid repeated freeze / thaw cycles.

Positive Control: #0038: phosphotyrosine MW standard

Immunoblotting: 0.5 µg/ml for HRPO/ECL detection
Recommended blocking buffer: Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.

Immunoprecipitation: use at 1 - 10 µg per 10⁶ pervanadate-treated A431 cells

Immunocytochemistry: ND

ELISA: use at 0.05 µg/ml

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Immunoblotting Reagents

Phosphotyrosine Molecular Weight Marker

Order No.: 0038/pTyr-MW-S
Lot: 0038
Size (ml) 20 Blots



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orders & support:

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03/080507F

Formulation

Phosphotyrosine modified standard proteins lyophilized from PBS/0.1% SDS/PEG/Sucrose/Malachitgreen.

The following standard proteins were modified with phosphotyrosine: galactosidase (116kD), phosphorylase A (98kD), BSA (67kD), ovalbumin (46kD), carbonic anhydrase (32kD), and soybean trypsin inhibitor (24kD).

Storage

Reconstitute by addition of 200 µl H₂O. After complete solubilization add 200 µl 2x SDS-PAGE sample buffer, mix and incubate at 90°C for 5 min.

Application

The phosphotyrosine molecular weight marker is recommended for immunoblot applications. Use 20µl of the phosphotyrosine molecular weight marker per lane (mini gel).

The individual proteins of the marker are recognized by the following commercially available monoclonal antibodies:

2C8, 1F9, 3B12, 9F1, 9H8, 16F4 (nanoTools)
4G10 (Upstate/Millipore)
PY20 (BD)
PT66 (Sigma)