

# Phosphotyrosine Detection Kit

Order No.: **0703/PTYR-KIT**



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.:** 0030S



02/060307F

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

<b>Purification:</b>	The antibody was purified from serum-free cell culture supernatant by subsequent ultrafiltration and size exclusion chromatography.
<b>Formulation:</b>	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
<b>Reconstitution:</b>	Reconstitute with 1 ml H <sub>2</sub> O (15 min, RT).
<b>Stability:</b>	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.
	<b>Avoid repeated freeze / thaw cycles.</b>
<b>Positive Control:</b>	#0038: phosphotyrosine MW standard
<b>Immunoblotting:</b>	0.5 µg/ml for HRPO/ECL detection <b>Recommended blocking buffer:</b> Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
<b>Immunoprecipitation:</b>	use at 1 - 10 µg per 10 <sup>6</sup> pervanadate-treated A431 cells
<b>Immunocytochemistry:</b>	ND
<b>ELISA:</b>	use at 0.05 µg/ml

**All products are supplied for research and investigational use only. Not for use in humans or laboratory animals.**

## Mouse Monoclonal Antibody to

# Phosphotyrosine

## clone 2A5

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

**Size (µg)** 25

**Lot No.:** 0133S



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

<b>Purification:</b>	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
<b>Formulation:</b>	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
<b>Reconstitution:</b>	Reconstitute with 1 ml H <sub>2</sub> O (15 min, RT).
<b>Stability:</b>	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.
	<b>Avoid repeated freeze / thaw cycles.</b>
<b>Positive Control:</b>	#0038: phosphotyrosine MW standard
<b>Immunoblotting:</b>	0.5 µg/ml for HRPO/ECL detection <b>Recommended blocking buffer:</b> Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
<b>Immunoprecipitation:</b>	use at 1 - 10 µg per 10 <sup>6</sup> pervanadate-treated A431 cells
<b>Immunocytochemistry:</b>	ND
<b>ELISA:</b>	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



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

<b>Purification:</b>	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
<b>Formulation:</b>	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
<b>Reconstitution:</b>	Reconstitute with 1 ml H <sub>2</sub> O (15 min, RT).
<b>Stability:</b>	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.

<b>Positive Control:</b>	#0038: phosphotyrosine MW standard
<b>Immunoblotting:</b>	0.5 µg/ml for HRPO/ECL detection <b>Recommended blocking buffer:</b> Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.

<b>Immunoprecipitation:</b>	use at 1 - 10 µg per 10 <sup>6</sup> pervanadate-treated A431 cells
<b>Immunocytochemistry:</b>	ND
<b>ELISA:</b>	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



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<sub>2</sub>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<sup>6</sup> 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



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

<b>Purification:</b>	The antibody was purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography.
<b>Formulation:</b>	lyophilized from 1 ml 2 x PBS / 0.09 % Na-azide / PEG and Sucrose.
<b>Reconstitution:</b>	Reconstitute with 1 ml H <sub>2</sub> O (15 min, RT).
<b>Stability:</b>	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.
	<b>Avoid repeated freeze / thaw cycles.</b>
<b>Positive Control:</b>	#0038: phosphotyrosine MW standard
<b>Immunoblotting:</b>	0.5 µg/ml for HRPO/ECL detection <b>Recommended blocking buffer:</b> Casein/Tween 20 based blocking and blot incubation buffer, e.g. nanoTools product #3031-500/CPPT or #3031-3000/CPPT.
<b>Immunoprecipitation:</b>	use at 1 - 10 µg per 10 <sup>6</sup> pervanadate-treated A431 cells
<b>Immunocytochemistry:</b>	ND
<b>ELISA:</b>	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



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 H<sub>2</sub>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<sup>6</sup> 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



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 H<sub>2</sub>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<sup>6</sup> 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



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 H<sub>2</sub>O. 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)