



Phosphothreonine Detection Kit

Order No.:

0702/PTHR-KIT

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orders & support: email: info@nanotools.de phone: +49-7641-455 670 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 assembley 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 Phosphothreonine Detection Kit contains 3 different phosphotyrosine specific monoclonal antibodies.

Do not use Milk or Casein based blocking and incubation buffers.

| clone | isotype | order number |
|-------|---------|--------------|
| 1E11 | lgG1 | 0024-025 |
| 4D11 | IgM | 0025-025 |
| 14B3 | lgG1 | 0026-025 |

Postive control

This product contains the following positive control for immunoblot applications: #0901-PSRECO phosphoproteins from rabbit muscle



Mouse Monoclonal Antibody to

Phosphothreonine

clone 1E11

| CIOI | | | | | | fax: | +49-7641-455 670 | | |
|--|--|---|---|---|--------------------|--|--|--|--|
| Order Size (μι Lot No. | g) | 0024-02 25 0024S | 5/PTHR-1E11 | | | 03/1603 | 307F | | |
| lsotype | Species Reactiv | vity Applicatio | ons Mol. Weight | Ref.Cell Line | Epitope | | Immunogen | | |
| lgG1 | human, mouse, dog | rat, WB, ELISA | A, IP pattern | | | | phosphothreonine conjugated to KLH | | |
| Backgroun | nd and Specificity | <u>/:</u> | | | | Related Products | | | |
| extracellular the assemb modulating Modification Mab PTHR- | r signals to the cell ly of protein compl enzymatic activity n of proteins on sel -1E11 recognizes | I nucleus. Phosp lexes or may alter or the ability to u rine residues is n a broad range of | horylated epitopes or the 3-dimensiona undergo protein-prot nediated by serine/t | hreonine kinases. rylated proteins in ci | ng sites for us | #0018-100/pSer- #0019-100/pSer- #0020-100/pSer- #0021-100/pSer- #0022-100/pSer- #0023-100/pSer- | 4A3 4A9 4H4 7F12 16B4 • Phosphothreonine 4D11 | | |
| Purificatior | 5 | | | m-free cell culture c adsorption and size | 9 | | | | |
| Formulatio | | lyophilized from ´ Sucrose. | I ml 2 x PBS / 0.09 | % Na-azide / PEG a | Ind | | | | |
| Reconstitu | tion: | Reconstitute with | $1 \text{ ml H}_2\text{O}$ (15 min, | RT). | | | | | |
| Stability: | - | Upon reconstituti reconstituted ant Thaw aliquots at 1 week. | on, aliquote and fre | lizate upon arrival (-2 eze in liquid nitroger l frozen at -80°C up uots may be stored a | n; to 1 year. | | | | |
| | | - | - | | | | | | |
| Positive Co Immunoblo | otting: | 1 μg/ml for HRP0 <u>Recommended</u> blocking buffer. | D/ECL detection | onine positive contro SA/Tween 20 based OR BLOCKING! | | | | | |
| lmmunopre Immunocyt ELISA: | tochemistry: | use at 1 - 10 µg ND use at 0.05 µg/m | - - | e-treated A431 cells | | | | | |

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



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Mouse Monoclonal Antibody to

Phosphothreonine

| clor | ne 4D11 | | | | 88 | | info@nanotools.de +49-7641-455 670 +49-7641-455 671 |
|--|--|---|---|--|--------------------|--|---|
| Order No.: Size (μg) Lot No.: | | 0025-025 25 0025S | | | | | |
| | | | | | 000 | 03/1603 | |
| Isotype | Species Reactiv | | | Ref.Cell Line | Epitope | | Immunogen |
| IgM | dog | rat, WB, ELISA, | IP pattern | | | | phosphopeptide conjugated to KLH |
| Backgrour | nd and Specificity | <u>/:</u> | | | | Related Pr | oducts |
| extracellula the assemb modulating Modification | r signals to the cell ly of protein comp enzymatic activity of proteins on se -4D11 recognizes | Il nucleus. Phosph lexes or may alter or the ability to un rine residues is ma | orylated epitopes r the 3-dimensional dergo protein-prot ediated by serine/th preonine-phosphor | nreonine kinases. rylated proteins in cl | ng sites for us | #0018-100/pSer #0019-100/pSer #0020-100/pSer #0021-100/pSer #0022-100/pSer #0023-100/pSer | 4A3 4A9 4F12 16B4 Phosphothreonine 1E11 |
| Purification | : | The antibody was supernatant by sul exclusion chromat | osequent thiophilic | n-free cell culture adsorption and size | e | | |
| Formulatio | | lyophilized from 1 Sucrose. | ml 2 x PBS / 0.09 9 | % Na-azide / PEG a | ind | | |
| Reconstitu | tion: | Reconstitute with | I ml H_2O (15 min, F | RT). | | | |
| Stability: | | Upon reconstitutio reconstituted antib | n, aliquote and free ody can be stored | izate upon arrival (-2 eze in liquid nitroger frozen at -80°C up lots may be stored a | n; to 1 year. | | |
| | | Avoid repeated fi | eeze / thaw cycle | s. | | | |
| Positive Co | ontrol: | #0901: phosphose | rine/phosphothreo | nine positive contro | I | | |
| Immunoblo | - | 1 μg/ml for HRPO <u>Recommended b</u> blocking buffer. DO NOT USE MIL | locking buffer: B | SA/Tween 20 based DR BLOCKING! | 1 | | |
| Immunopre | ecipitation: | use at 1 - 10 µg p | er 10 ⁶ pervanadate | -treated A431 cells | | | |
| - | - | ND | | | | | |
| ELISA: | - | use at 0.05 µg/ml | | | | | |

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Mouse Monoclonal Antibody to

Phosphothreonine

clone 14B3

| 0101 | | | | | | 88 | fax: | +49-7641-455 671 |
|---|----------------------|--------------------------|-------------|-----------------------------|--|---------|--|--|
| Orde | r No.: | 0026 | -025/PT | THR-14B3 | | Ö | | |
| Size (µ | g) | 25 | | | | 80 | | |
| Lot No. | .: | 0026S | | | | | 03/1603 | 07F |
| Isotype | Species Reactiv | ity Appli | cations | Mol. Weight | Ref.Cell Line | Epitope | | Immunogen |
| lgG1 | human, mouse, dog | rat, WB, E | ELISA, IP | pattern | | | | phosphopeptide conjugated to KLH |
| Backgrour | nd and Specificity | <u>':</u> | | | | | Related Pro | oducts |
| 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 serine residues is mediated by serine/threonine kinases. Mab PTHR-14B3 recognizes a broad range of threonine-phosphorylated proteins in crude cell extracts, providing a valuable tool for non-radioactive phosphothreonine detection. | | | | | | | #0018-100/pSer-1 #0019-100/pSer-4 #0020-100/pSer-4 #0021-100/pSer-4 #0022-100/pSer-7 #0023-100/pSer-1 | A3 A9 F12 6B4 Phosphothreonine E11 |
| Purificatio | : | | by subsec | quent thiophilic a | i-free cell culture adsorption and size | 3 | | |
| Formulatio | | yophilized f Sucrose. | rom 1 ml 2 | 2 x PBS / 0.09 % | % Na-azide / PEG a | Ind | | |
| Reconstitu | ition: | Reconstitute | e with 1 ml | H₂O (15 min, R | T). | | | |
| 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 1 week. | | | | | | | | |
| | | Avoid repe | ated freez | e / thaw cycles | 5. | | | |
| Positive Co | ontrol: | #0901: phos | sphoserine | e/phosphothreor | nine positive contro | I | | |
| Immunoblo | | blocking but | ided bloc | | A/Tween 20 basec | 1 | | |
| Immunopre | ecipitation: | use at 1 - 10 |) ug per 1 | 0 ⁶ pervanadate- | treated A431 cells | | | |
| - | - | | | | | | | |
| ELISA: | - | use at 0.05 | µg/ml | | | | | |

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pSer / pThr Molecular Weight
Markerwww.nanotools.de
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email: info@nanotools.de
phone: +49-7641-455 670
fax: +49-7641-455 671Order No.:0901/PSERCOLot:0901Size20 Blots

Formulation The pSer/pThr molecular weight marker contains rabbit muscle phosphoproteins isolated by Fe3+/IDA - affinity chromatography. Proteins are lyophilized from PBS/NaF/PEG/Sucrose/ Bromo-phenolblue and Na - azide. After reconstitution the solution contains 0.09% Na-azide.

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

Aliquote and store frozen. Avoid repeated freeze/thaw cycles.

ApplicationThe pSer/pThr molecular weight marker is recommended for
immunoblot applications. Use 20µl molecular weight marker per lane.Note: Use BSA based blot incubation buffers. Milk. Casein and Blotto.

Note: Use BSA based blot incubation buffers. Milk, Casein and Blotto might interfere with antibody - antigen interaction.