Phosphoserine detection set

The kit offers a set of six different MAbs specific for serine phosphorylation sites. Phosphorylation patterns in a given cell extract may differ when probed with different antibodies due to sequence specificity. Recognition therefore depends on two criteria: phosphorylation and surrounding aa motif. If one of the two criteria is not fulfilled, the antibody will not detect the phosphorylation site. Specificity has been determined by epitope mapping using degenerated phosphopeptide libraries. Clone 16B4 strongly interacts with pSK and pSP motifs, thus specifically recognizes substrates of MAP/SAP Kinases and CDC kinases.Clones 1C8, 4A3, 4A9, 4H4 and 7F12 interact with epitopes containing phosphoserine in the context of positive or neutral aa (e.g. substrates of PKA, PKB, PKC, PKG etc.) Though revealing a similar pattern in epitope mapping using phosphopeptide libraries, these antibodies interact with different sets of phosphoproteins in whole cell lysates.

Antibodies directed 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.

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Ordering Information

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ALX-850-0230	1Set

Manuals, SDS & CofA

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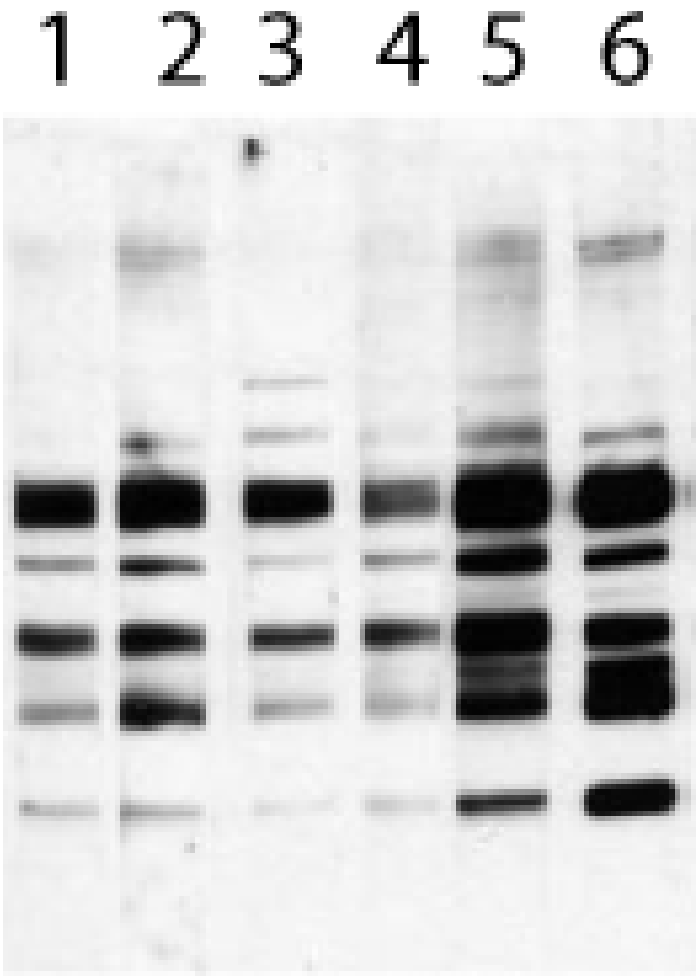


Figure: Phosphoprotein positive control was probed with 1µg/ml of the following MAbs: Lane 1: Clone 1C8 (Prod. No. ALX-804-162) Lane 2: Clone 4A3 (Prod. No. ALX-804-163) Lane 3: Clone 4A9 (Prod. No. ALX-804-164) Lane 4: Clone 4H4 (Prod. No. ALX-804-165) Lane 5: Clone 7F12 (Prod. No. ALX-804-166) Lane 6: Clone 16B4 (Prod. No. ALX-804-167).

Handling & Storage

Use/Stability	As indicated on product label or CoA when stored as recommended.
Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	IP, WB
Contents	Contains a positive control for immunoblot applications as well as 25µg each of the following MAbs: Clone 1C8 (Prod. No. ALX-804-162) Clone 4A3 (Prod. No. ALX-804-163) Clone 4A9 (Prod. No. ALX-804-164) Clone 4H4 (Prod. No. ALX-804-165) Clone 7F12 (Prod. No. ALX-804-166) Clone 16B4 (Prod. No. ALX-804-167)
Recommendation Dilutions/Conditions	Western blot (20µl/lane (mini gel) for HRPO/DAB detection, or 5µl/lane (mini gel) for HRPO/ECL detection. Do not use milk/casein for blocking and dilution! Replace by BSA! We recommend to use 1% BSA, 1% PVP-10 (polyvinyl-pyrrolidone)1% PEG 3500, 0.2% Tween 20 in 2x PBS)Suggested dilutions/conditions may not be available for all applications.Optimal conditions must be determined individually for each application.

Technical Info / Product Notes

Antibodies directed 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.

Positive Control Cell Lysate

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 and Bromophenolblue.

Reconstitution: Reconstitute by addition of 200 µl H2O. After complete solubilization add 200 µl 2x SDS-PAGE sample buffer, mix and incubate at 90ºC for 5 min. Aliquote and store frozen. Avoid repeated freeze/thaw cycles.

Application: The 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 might interfere with antibody – antigen interaction.

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