

Affinity-Purified Rabbit Anti-phospho-INS R (Y1162/Y1163)/IGF-I R (Y1135/Y1136) Antibody

ORDERING INFORMATION

Catalog Number: AF2507

Lot Number: UT103

Size: 50 µg (sufficient for 100 mL of blotting solution)

Storage: -20° C

Specificity: human phospho-INS R (Y1162/Y1163) and IGF-1 R (Y1135/Y1136)

Immunogen: phosphopeptide containing human INS R Y1162/Y1163 sites

Ig Type: rabbit IgG

Application: Western blot
Flow cytometry

Background

The heterotetrameric receptors for insulin (INS R) and IGF-I (IGF-I R) are receptor tyrosine kinases that consist of two ligand-binding α subunits and two β subunits. Ligand binding induces autophosphorylation on multiple tyrosine residues of β subunits. Phosphorylation of Tyr 1162 and 1163 on INS R and Tyr 1135 and 1136 on IGF-I R stimulates intrinsic kinase activity.

Preparation

Rabbit antibodies were raised against a synthetic phosphopeptide corresponding to residues surrounding Y1162 and Y1163 of human INS R. This phosphopeptide is identical to the sequence surrounding Y1135 and Y1136 of human IGF-I R. Polyclonal antibody was affinity-purified on a column derivatized with the phosphopeptide, and further purified by protein A chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects endogenous human INS R dually phosphorylated at Y1162 and Y1163, and human IGF-I R dually phosphorylated at Y1135 and Y1136. Reactivity with other species has not been determined.

Applications

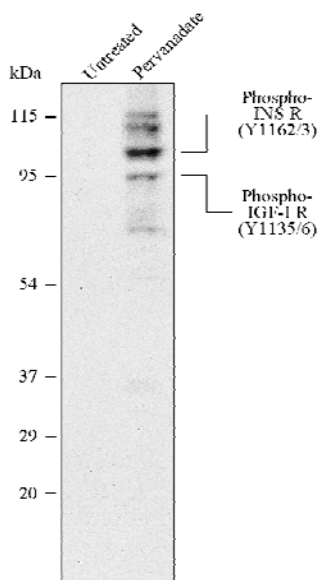
Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Protocols for Western blotting

<u>Blotting Buffer</u>	<u>Blocking Solution</u>	<u>Antibody Solution</u>
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Antibody Solution containing 0.5 µg/mL rabbit anti-phospho-INS R (Y1162/Y1163).
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-rabbit IgG (R&D Systems, Catalog # HAF008).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell Lysates for Western blotting - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10⁶ - 1 x 10⁷ cells per mL.

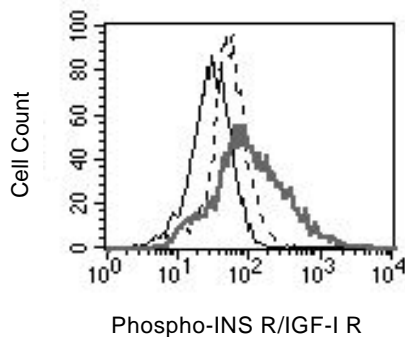


Detection of phosphorylated INS R and IGF-1 R with AF2507. Human A431 cells were either untreated or treated with 100 µM Pervanadate and harvested 10 minutes after stimulation. Total cell lysates in gel sample buffer were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 0.5 µg/mL rabbit anti-phospho-INS R (Y1162/Y1163), as described in Protocols for Immunoblotting. A 30 second exposure to film is shown.

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R&D Systems, Inc.
1-800-343-7475

Flow cytometry - For intracellular staining to detect phospho-INS R (Y1162/Y1163)/IGF-I R (Y1135/Y1136), cells must first be fixed and permeabilized using 2% paraformaldehyde and ice-cold methanol. Dilute this antibody to 10 µg/mL and add 10 µL of the diluted solution to 1 - 5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. Following a 30 minute incubation, cells should be washed in an isotonic phosphate buffer (supplemented with 0.5% BSA) prior to the addition of a secondary developing reagent. The binding of unlabeled polyclonal antibodies may be visualized by adding 10 µL of a 25 µg/mL solution of a secondary developing reagent such as anti-rabbit IgG conjugated to a fluorochrome. Cells should be washed for a final time prior to flow cytometric analysis.



Control (dashed line) or pervanadate-treated human HeLa cells (thick line) were stained with anti-phospho-INS R/IGF-I R antibody (R&D Systems, Catalog # AF2507) or control antibody (R&D Systems, Catalog # AB-105-C, thin line), followed by PE-conjugated anti-rabbit antibody.

Optimal dilutions should be determined by each laboratory for each application.

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