Human Phospho-Insulin R (Y1162/Y1163)/ IGF-I R (Y1135/Y1136) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG

Catalog Number: AF2507

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Insulin R dually phosphorylated at Y1162 and Y1163, and human IGF-I R dually phosphorylated at Y1135 and Y1136.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human Insulin R Y1162/Y1163 sites
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

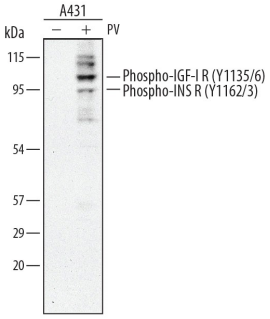
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

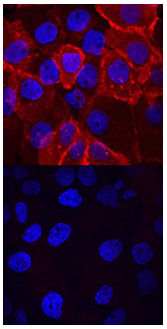
DATA

Western Blot



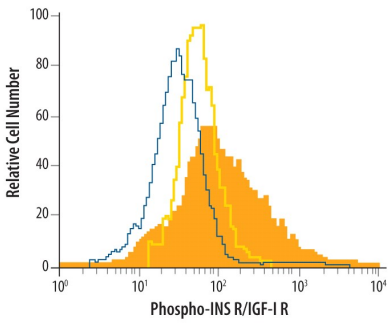
Detection of Human Phospho-Insulin R (Y1162/Y1163) and Phospho-IGF-I R (Y1135/1136) by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line untreated (-) or treated (+) with 100 µM pervanadate (PV) for 10 minutes. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human Phospho-Insulin R (Y1162/Y1163)/IGF-I R (Y1135/Y1136) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2507), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Specific bands were detected for Phospho-Insulin R (Y1162/Y1163) at 95 kDa and Phospho-IGF-I R (Y1135/1136) at approximately 100 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Phospho-Insulin R (Y1162/1163)/IGF-I R (Y1135/1136) in A431 Human Cell Line. Insulin R phosphorylated at Y1162/1163 and IGF-I R phsophorylated at Y1135/1136 were detected in immersion fixed A431 human epithelial carcinoma cell line untreated (lower panel) or treated (upper panel) with pervanadate using Rabbit Anti-Human Phospho-Insulin R (Y1162/1163)/IGF-I R (Y1135/1136) Cross-reactive Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2507) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Intracellular Staining by Flow Cytometry



Detection of Insulin R/CD220 in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was unstimulated (light orange open histogram) or treated with 100 µM pervanadate for 15 minutes, then stained with Rabbit Anti-Human Phospho-Insulin R (Y1162/1163)/IGF-I R (Y1135/1136) cross-reactive Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2507, dark orange filled histogram) or isotype control antibody (Catalog # AB-105-C, blue open histogram), followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with methanol.

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Human Phospho-Insulin R (Y1162/Y1163)/ IGF-I R (Y1135/Y1136) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF2507

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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 Tyr1162 and 1163 on INS R and Tyr1135 and 1136 on IGF-I R stimulates intrinsic kinase activity.

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