Store at -20°C

#8338

Phospho-Insulin/IGF Receptor Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
IGF-I Receptor β (D23H3) XP® Rabbit mAb	9750	20 µl	95 kDa	Rabbit IgG
Phospho-IGF-I Receptor β (Tyr1135) (DA7A8) Rabbit mAb	3918	20 µl	95 kDa	Rabbit IgG
Phospho-IGF-I Receptor β (Tyr1131)/Insulin Receptor β (Tyr1146) Antibody	3021	20 µl	95 kDa	Rabbit IgG
Phospho-IGF-I Receptor β (Tyr1135/1136)/Insulin Receptor β (Tyr1150/1151) (19H7) Rabbit mAb	3024	20 µl	95 kDa	Rabbit IgG
Phospho-IGF-I Receptor β (Tyr980) (C14A11) Rabbit mAb	4568	20 µl	95 kDa	Rabbit IgG
Insulin Receptor β (4B8) Rabbit mAb	3025	20 µl	95 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

U.S. Patent No. 5,675,063

Description: The Phospho-Insulin/IGF Receptor Antibody Sampler Kit provides an economical means of evaluating total Insulin Receptor and IGF-I Receptor β protein levels as well as Insulin and IGF-I Receptor β phosphorylated at specific sites. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Type I insulin-like growth factor receptor (IGF-IR) is a transmembrane receptor tyrosine kinase that is widely expressed in many cell lines and cell types within fetal and postnatal tissues (1-3). Receptor autophosphorylation follows binding of the IGF-I and IGF-II ligands. Three tyrosine residues within the kinase domain (Tyr1131, Tyr1135, and Tyr1136) are the earliest major autophosphorylation sites (4). Phosphorylation of these three tyrosine residues is necessary for kinase activation (5,6). Insulin receptors (IRs) share significant structural and functional similarity with IGF-I receptors, including the presence of an equivalent tyrosine cluster (Tyr1146/1150/1151) within the kinase domain activation loop. Tyrosine autophosphorylation of IRs is one of the earliest cellular responses to insulin stimulation (7). Autophosphorylation begins with phosphorylation of Tyr1146 and either Tyr1150 or Tyr1151, while full kinase activation requires triple tyrosine phosphorylation (8).

Specificity/Sensitivity: Each antibody in the Phospho-Insulin/IGF Receptor Antibody Sampler Kit recognizes endogenous levels of the specific target protein, with activation state-specific antibodies recognizing target proteins only when phosphorylated at the indicated residues. IGF-I Receptor β (D23H3) XP® Rabbit mAb detects endogenous levels of total IGF-I receptor β protein. This antibody does not cross-react with insulin receptor.Phospho-IGF-I Receptor β (Tyr1135) (DA7A8) Rabbit mAb detects endogenous levels of IGF-I receptor only when phosphorylated at Tyr1135. This antibody cross-reacts with Tyr1150 of insulin receptor and may also cross-react with other overexpressed related tyrosine-phosphorylated tyrosine kinases. Phospho-IGF-I Receptor β (Tyr1131)/Insulin Receptor β (Tyr1146) Antibody detects endogenous levels of Tyr1131-phosphorylated IGF-I receptor and Tyr1146-phosphorylated insulin receptor. This antibody cross-reacts with activated PDGF, FGF and EGF receptors; ErbB2; and c-Met. Phospho-IGF-I Receptor β (Tyr1135/1136)/Insulin Receptor β (Tyr1150/1151) (19H7) Rabbit mAb detects endogenous levels of IGF-I receptor and insulin receptor only when phosphorylated at tyrosine 1135/1136 or tyrosine 1150/1151, and does not cross-react with other related tyrosine-phosphorylated tyrosine kinases. Phospho-IGF-I Receptor β (Tyr980) (C14A11) Rabbit mAb detects endogenous levels of IGF-I receptor β protein only when phosphorylated at Tyr980, and may cross-react with activated insulin receptors and FLT3. Insulin Receptor β (4B8) Rabbit mAb detects endogenous levels of total insulin receptor β and does not cross-react with IGF-I receptor β.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy-terminal residues of human IGF-IR β or residues surrounding Tyr960 of human insulin receptor β. Activation state-specific monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr1135 of human IGF-I Receptor β, Tyr1135/1136 of human IGF-I Receptor β, or Tyr980 of human IGF-I Receptor β. Activation state-specific polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues of human IGF-I Receptor β. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Adams, T.E. et al. (2000) *Cell. Mol. Life Sci.* 57, 1050-1093.
(2) Baserga, R. et al. (2000) *Oncogene* 19, 5574-5581.
(3) Scheidegger, K.J. et al. (2000) *J. Biol. Chem.* 275, 38921-38928.
(4) Hernandez-Sanchez, C. et al. (1995) *J. Biol. Chem.* 270, 29176-29181.
(5) Lopaczynski, W. et al. (2000) *Biochem. Biophys. Res. Commun.* 279, 955-960.
(6) Baserga, R. et al. (1999) *Exp. Cell Res.* 253, 1-6.
(7) White, M.F. et al. (1985) *J. Biol. Chem.* 260, 9470-9478.
(8) White, M.F. et al. (1988) *J. Biol. Chem.* 263, 2969-2980.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#8338

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween®20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.