Store at 4°C

#7828

PathScan® Phospho-Insulin Receptor β (Tyr1150/1151) Sandwich ELISA Antibody Pair

☑ 1 Kit
(4 X 96 assays)

Cell Signaling TECHNOLOGY®

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

Species Cross-Reactivity: H, M

Description: CST's PathScan® Phospho-Insulin Receptor β (Tyr1150/1151) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Insulin Receptor β (Tyr1150/1151) Sandwich ELISA Kit #7258. Capture and detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The insulin receptor β capture antibody is coated on a 96 well microplate in PBS overnight. After blocking, cell lysates are added followed by a phospho-insulin receptor β (Tyr1150/1151) detection antibody and anti-rabbit IgG, HRP conjugated antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-insulin receptor β (Tyr1150/1151) protein.

Reagents not supplied:
Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween®20 (PBST-20X) #9809

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween®20, 1% BSA

96 Well Microplates**

Microplate Reader

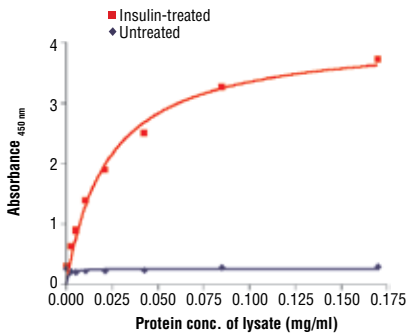
** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

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 insulin receptor 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).

Product Includes	Item #	Volume	Cap Color	Storage Temp
Insulin Receptor β Capture Mouse mAb (100X)	96148	400 µL	Pink	4°C
Phospho-Insulin Receptor (Tyr1150/1151) Detection Rabbit mAb (100X)	86202	400 µL	Blue	4°C
Anti-rabbit IgG, HRP-linked Antibody (1000X)	25944	40 µL	Red	–20°C



The relationship between protein concentration of lysates from untreated or insulin-treated CHO-IR/IRS-1 cells and the absorbance at 450 nm using PathScan® Phospho-Insulin Receptor β (Tyr1150/1151) Sandwich ELISA Antibody Pair #7828 is shown. CHO-IR/IRS-1 cells (85% confluence) were serum starved overnight and then treated with insulin (100 nM) for 2 min at 37°C, and then lysed.

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

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**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween®20, (20X PBST #9809)
3. **Blocking Buffer:** 1X PBS/0.05% Tween®20, 1% BSA
4. **1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)
   20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin.
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with Wash Buffer, 200 µl each time for each well. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   c. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

1. Aspirate media, treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.