

Store at 4°C and
-20°C
#7823**PathScan® Phospho-Insulin Receptor β
(Tyr1345) Sandwich ELISA Antibody Pair****Orders:** 877-616-CELL (2355)
orders@cellsignal.com**Support:** 877-678-TECH (8324)**Web:** info@cellsignal.com
cellsignal.com**Species Cross Reactivity:** H M
UniProt ID: #P06213
Entrez-Gene ID: #3643

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For Research Use Only. Not for Use in Diagnostic Procedures.**Description**

CST's PathScan® Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Kit #7326. 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 phospho-insulin receptor β (Tyr1345) capture antibody is coated on a 96 well microplate in PBS overnight. After blocking, cell lysates are added followed by an insulin receptor β detection antibody and anti-mouse 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 (Tyr1345) protein.

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 at Tyr1146 and either Tyr1150 or Tyr1151, while full kinase activation requires triple tyrosine phosphorylation (8).

Background References

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5. Lopaczynski, W. et al. (2000) *Biochem Biophys Res Commun* 279, 955-60.
6. Baserga, R. (1999) *Exp Cell Res* 253, 1-6.
7. White, M.F. et al. (1985) *J Biol Chem* 260, 9470-8.
8. White, M.F. et al. (1988) *J Biol Chem* 263, 2969-80.

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#7823

PathScan[®] Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Antibody Pair

ELISA Antibody Pair

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
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): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH₂O, mix. Buffer can be stored at 4°C for short-term use (1-2 weeks).

Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **Bovine Serum Albumin (BSA):** (#9998).
6. **TMB Substrate:** (#7004).
7. **STOP Solution:** (#7002)

NOTE: Reagents should be made fresh daily.

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80-90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) 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.

For suspension cells

1. Remove media by low speed centrifugation (~1,200 rpm) when the culture reaches 0.5-1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5-10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) 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.

C. Coating Procedure

1. Rinse microplate with 200 μ l of dH₂O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 μ l of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 μ l/well. Cover plate and incubate overnight at 4°C (17-20 hr).
3. **After overnight coating, gently uncover plate and wash wells:**
 1. Discard plate contents into a receptacle.
 2. Wash four times with wash buffer, 200 μ l each time per well. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 μ l of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100 μ l of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100 μ l of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100 μ l/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10 μ l of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100 μ l/well. Cover and incubate at 37°C for 30 min.
6. Wash plate (Section C, Step 3).
7. Add 100 μ l of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100 μ l of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
 1. **Visual Determination:** Read within 30 min after adding STOP solution.
 2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

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