Revision 1

Store at 4°C

#7326

PathScan® Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Kit

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Species Cross Reactivity	UniProt ID:	Entrez-Gene Id:
H M	#P06213	#3643

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Color	Storage Temp
Insulin Receptor ß Mouse Detection mAb	13741	1 ea	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 ea	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml	Colorless	4°C
STOP Solution	7002	11 ml	Colorless	4°C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Sealing Tape	54503	2 ea		4°C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20°C

*The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

CST's PathScan® Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects transfected phospho-insulin receptor (Tyr1345) protein. Insulin Receptor β (Tyr1345) Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, only phospho-insulin receptor proteins are captured by the coated antibody. Following extensive washing, Insulin Receptor β Mouse mAb is added to detect the captured phospho-insulin receptor β (Tyr1345) protein. Anti-mouse IgG, HRP-linked Antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of phospho-insulin receptor (Tyr1345) protein. Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

CST's PathScan® Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Kit #7326 detects phospho-insulin receptor (Tyr1345) protein. As shown in Figure 1, using Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Kit #7326, a significant induction of phospho-insulin receptor (Tyr1345) is detected in CHO-IR/IRS-1 cells treated with insulin. The levels of total insulin receptor β (phospho and nonphospho) shown by Western analysis remain unchanged. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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

1. Adams, T.E. et al. (2000) *Cell Mol Life Sci* 57, 1050-93.
2. Baserga, R. (2000) *Oncogene* 19, 5574-81.
3. Scheidegger, K.J. et al. (2000) *J Biol Chem* 275, 38921-8.
4. Hernández-Sánchez, C. et al. (1995) *J Biol Chem* 270, 29176-81.
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.

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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 **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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#7326

PathScan® Phospho-Insulin Receptor β (Tyr1345) Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

ELISA Colorimetric

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature.

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 PBS: add 50 ml 10X PBS to 950 ml dH_2O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH_2O.
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 dH_2O, 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.

   NOTE: Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate**: (#7004).
6. **STOP Solution**: (#7002).

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 10^6 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. Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed in the storage bag and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with sample diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
4. **Gently remove the tape and wash wells**:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X wash buffer, 200 µl each time per well.
    3. 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.
    4. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of detection antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100 µl of STOP solution to each well. Shake gently for a few seconds.

    NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP solution.

11. **Read results**
    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.

posted June 2005

revised November 2013