Store at -80°C

#7748

INS Receptor Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

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Description: Purified recombinant human INSR kinase (Gly989-Ser1382), supplied as a GST fusion protein.

Background: Insulin receptor (INSR) is a membrane receptor tyrosine kinase. The receptor molecule consists of a disulfide linked heterodimer. The α subunit is a 135 kDa extracellular fragment, and the beta subunit is 95 kDa fragment containing an extracellular domain, a single transmembrane domain, and an intracellular tyrosine kinase domain (1). Insulin ligand binding to this receptor results in receptor autophosphorylation and tyrosine kinase activation. INSR catalyses the tyrosine phosphorylation of molecules such as IRS, Gab-1, Shc and Cbl, which further activate the down stream MAPK, PI3K, TC10 pathway and eventually lead to increases in glucose uptake and metabolism as well as cell growth (2,3). INSR has peptide substrate specificity similar to other receptor tyrosine kinase members, prefering acidic residues at the -1 to -4 positions and large hydrophobic amino acids at positions +1 and +3 (4).

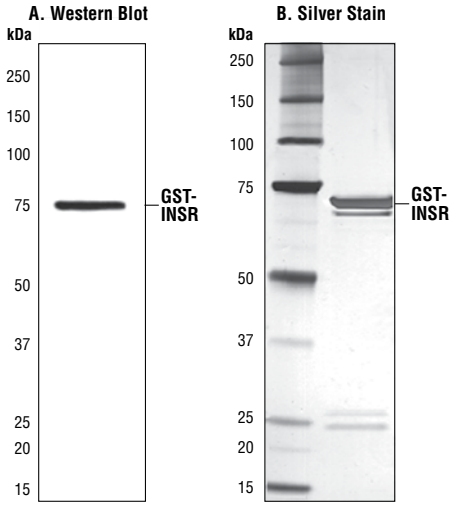

Figure 1. The purity of the GST-INSR fusion protein was analyzed using SDS/PAGE followed by anti-INSR Western blot (A) or Silver stain (B).

Source/Purification: The GST Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human INSR (Gly989-Ser1382) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-INSR kinase fusion protein is 70.4 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. INSR kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure INSR activity using HTScan™ INS Receptor Kinase Assay Kit #7430 [Fig.3].

Background References:

(1) Yip, C.C. and Ottensmeyer, P. (2003) *J. Biol. Chem.* 278, 27329–27332.

(2) Saltiel, A.R. and Pessin, J.E. (2002) *Trends Cell Biol.* 12, 65–71.

(3) Zick, Y. (2001) *Trends Cell Biol.* 11, 437–441.

(4) Songyang, Z. and Cantley, L.C. (1995) *TIBS* 20, 470–475.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at –80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ INSR Kinase Assay Kit #7430

IRS-1 (Tyr891) Biotinylated Peptide #1320

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

HTScan™ Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Staurosporine #9953

Tyrosine Kinase Substrate Screening Kit #7450

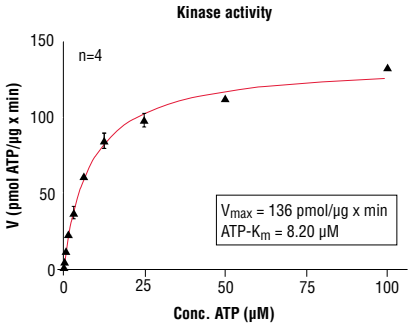

Figure 2. INSR kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: PolyAEKY, 10 µg/50 µl, Recombinant INSR: 25 ng/50 µl.

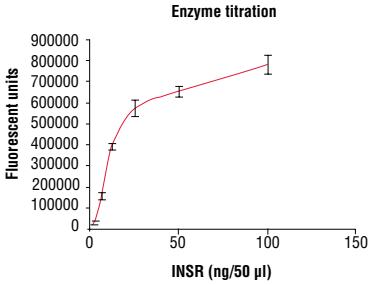

Figure 3. Dose dependence curve of INSR kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of peptide (#1320) by GST-INSR kinase. In a 50 µl reaction, increasing amounts of GST-INSR and 1.5 µM substrate peptide were used per reaction well at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for INS Receptor Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411
5. HTScan™ Tyrosine Kinase Buffer (4X) #9805
6. ATP (10 mM) #9804
7. IRS1 (Tyr891) Biotinylated Peptide #1320
8. DTT (1000X, 1.25 M)
9. DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
10. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
11. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Immediately transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 10 µl of DTT (1.25 M) to 2.5 ml of 4X HTScan™ Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3V0_4) to make DTT/Kinase buffer.
5. Transfer 1.25 ml of DTT/Kinase buffer to enzyme tube to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

60 mM HEPES pH 7.5
5 mM $MgCl_2$
5 mM $MnCl_2$
3 µM Na_3VO_4
1.25 mM DTT
200 µM ATP
1.5 µM peptide
50 ng INS Receptor Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction and 75 µl of dH_20/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a IRS1 (Tyr891) Biotinylated Peptide and Phospho-Tyrosine Mouse mAb (P-Tyr-100) diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled anti-mouse IgG 1:500 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com