SignalChem
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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
I08-11G -05	5 µg
I08-11G -10	10 µg
I08-11G -20	20 µg

InsR, Active

Recombinant human protein expressed in Sf9 cells

Catalog # I08-11G
Lot # W342-2

Product Description

Recombinant human InsR (1011-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_000208.

Gene Aliases

HHF5, CD220

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

InsR is the insulin receptor tyrosine kinase that is involved in insulin signaling. InsR is post-translationally cleaved into two chains, α and β, that are covalently linked. Binding of insulin to the InsR stimulates glucose uptake (1). Insulin receptor signaling helps to maintain fuel homeostasis and prevent diabetes. Studies have shown that a conditional knockout of insulin receptor substrate 2 (IRS2) in mouse pancreas β cells and parts of the brain--including the hypothalamus--increased appetite, lean and fat body mass, linear growth, and insulin resistance that progressed to diabetes. InsR signaling also increases the regeneration of adult β cells and the central control of nutrient homeostasis (2).

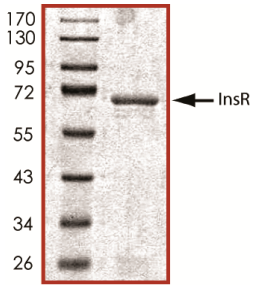
References

1. Okamoto, H. et al: Transgenic rescue of insulin receptor-deficient mice. J. Clin. Invest. 2004;114(2):214-23.
2. Lin, X. et al: Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. J. Clin. Invest. 2004;114(7):886-8.

Specific Activity

The specific activity of InsR was determined to be **2282 nmol /min/mg** as per activity assay protocol.

Purity



The purity of InsR was determined to be **>95%** by densitometry, approx. MW **70kDa**.

InsR, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	I08-11G
Specific Activity	2282nmol/min/mg
Specific Lot Number	W342-2
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: I08-11G)

Active InsR (0.1μg/μl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active InsR for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09) Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with final 50ng/μl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 20mM $MgC1_2$, 12.5mM $MnC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250μM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150μl of 10mM ATP Stock Solution (Catalog #: A50-09), 100μl [^{33}P]-ATP (1mCi/100μl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer II (Catalog #: K02-09). Store 200μl aliquots at –20°C.

Substrate (Catalog #: A16-58)

Axltide synthetic peptide substrate (KKSRGDYMTMQIG) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active InsR, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active InsR (Catalog #I08-11G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #A16-58)
- **Component 3.** 5μl distilled H_2O (4°C)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5 μl [^{33}P]-ATP Assay Cocktail bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P^{33}]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 μl [^{33}P]-ATP / pmoles of ATP (in 5 μl of a 250 μM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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