SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
I08-122G -05	5 µg
I08-122G -10	10 µg

InsR (R1201Q), Active

Recombinant human protein expressed in Sf9 cells

Catalog # I08-122G

Lot # H2882-10

Product Description

Recombinant human InsR (R1201Q) (980-1382) 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, and 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.

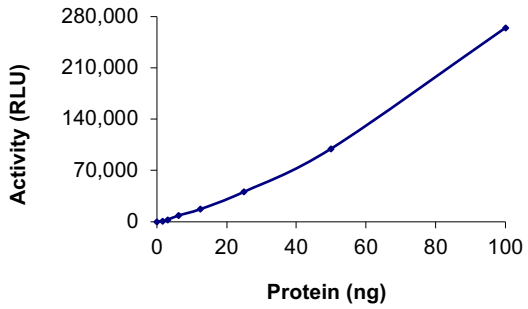
Scientific Background

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

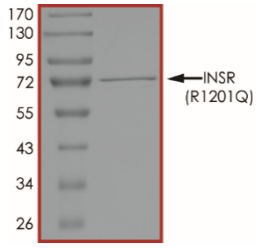
1. Okamoto, H. et al: Transgenic rescue of insulin receptor-deficient mice. J. Clin. Invest. 2004;114(2):214-23.
2. Lin, X. et al: Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. J. Clin. Invest. 2004;114(7):886-8.

Specific Activity



The specific activity of InsR (R1201Q) was determined to be **6.6 nmol/min/mg** as per activity assay protocol.

Purity



The purity of InsR (R1201Q) was determined to be **>90%** by densitometry, approx. MW **72 kDa**.

InsR (R1201Q), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	I08-122G
Specific Activity	6.6 nmol/min/mg
Lot #	H2882-10
Purity	>90%
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 #: I08-122G)
Active InsR (R1201Q) (0.1μg/μl) diluted with Kinase Dilution Buffer X (Catalog #: K20-09) 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 (R1201Q) for optimal results).

Kinase Assay Buffer III (5x) (Catalog#: K03-09)
Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Kinase Dilution Buffer X (Catalog#: K20-09)
Buffer components: 40mM Tris-HCl, pH 7.4, 20mM $MgCl_2$, 2.5 mM $MnCl_2$ and 0.1mg/mL BSA. Add fresh DTT prior to use to a final concentration of 50μM.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10mM
ADP solution, 10mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: A16-58)

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

Cofactor: 2.5M $MnCl_2$ (Catalog #: M40-09-25)
Diluted in distilled H_2O to a working concentration of 1M.

Assay Protocol

The KINASE assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the InsR (R1201Q) reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active InsR (R1201Q), Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15μL enzyme dilution with Kinase Dilution Buffer X at the desired concentration, in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25μM ATP example):

Component	Amount (μL)	Component	Amount (μL)
10mM ATP Solution	1	Substrate at 1mg/mL	80
Kinase Assay Buffer III (5x)	78	1M $MnCl_2$	1

Step 3. Transfer the following reaction components prepared in Steps 1 and 2 to a 384-well opaque plate, bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted Active InsR (R1201Q) (Catalog # I08-122G).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer X.

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

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

Calculation of Specific Activity of ADP (RLU/pmol)

From ATP-ADP conversion curve, determine RLU/pmol of ADP

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

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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MATERIAL SAFETY DATA SHEET

Article 1 - Product Identification and Use

Product Name: InsR (R1201Q), Active **Catalog # I08-122G**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name:	SignalChem Pharmaceuticals Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazardous Ingredients

NOT AVAILABLE. We are not aware of any hazards associated with this product or its ingredients, but the chemical, physical, and toxicological properties of this product have not been investigated thoroughly. Observe normal laboratory precautions.

Article 3 - Physical Data

This product consists of purified protein in Tris-HCl buffer shipped on dry ice. The physical properties of this product have not been investigated thoroughly.

Article 4 - Fire and Explosion Hazard

NOT APPLICABLE

Article 5 - Reactivity Data

NOT APPLICABLE

Article 6 – Toxicologically Data

May be harmful by inhalation, ingestion, or skin absorption. The toxicological properties of this product have not been investigated thoroughly. Exercise due caution.

Article 7 - Preventative Measures

Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

*****MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES*****

- Wear protective equipment.
- Absorb on sand or vermiculite and place in closed containers for disposal.
- Observe all federal, state and local environmental regulations.

Article 8 - First Aid Measures

- If swallowed, wash out mouth with water, provided person is conscious. Call a physician.
- In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, call a physician.
- If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.
- In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Call a physician.

Article 9 - Preparation

Prepared By: Mya Zhang Phone #: 1-866-954-6273