

IRR, Active

Recombinant protein expressed in Sf9 cells

Catalog # I07-11G

Lot # Q117-1

Product Description

Recombinant human IRR (945-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_014215](#).

Gene Aliases

INSRR

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 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.

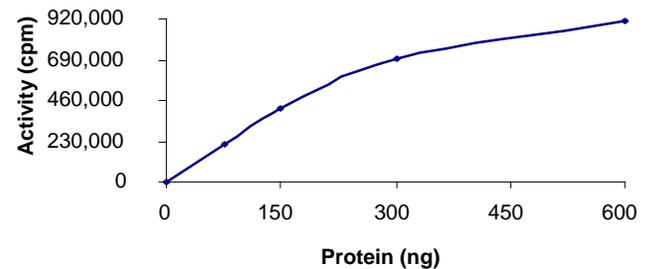
Scientific Background

IRR or insulin receptor-related receptor is a tyrosine protein kinase that is required for the appearance of male gonads and thus for male sexual differentiation (1). The expression of IRR mRNA is restricted to a few tissue types and has been detected in neuronal tissues as well as in neuroblastomas. As a result of its occurrence in neuroblastomas, it has speculated that IRR may be involved in tumor genesis. RT-PCR and solution hybridization/RNase protection assay have shown strong coupling of IRR mRNA in 18 tumor cell lines (2).

References

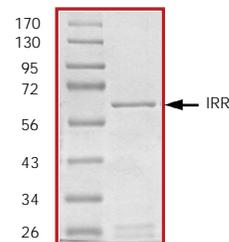
- Zhang, B. and Roth, R.A. The insulin receptor-related receptor. Tissue expression, ligand binding specificity, and signaling capabilities. *J. Biol. Chem.* 1992; 267 (26): 18320-8.
- Elmlinger, M.W. et al: Correlation of type I insulin-like growth factor receptor (IGF-I-R) and insulin receptor-related receptor (IRR) messenger RNA levels in tumor cell lines from pediatric tumors of neuronal origin. *Regul. Pept.*, 1999; 84 (1-3): 37-42.

Specific Activity



The specific activity of IRR was determined to be **111 nmol/min/mg** as per activity assay protocol.

Purity



The purity of IRR was determined to be **>90%** by densitometry, approx. MW **65kDa**.

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Specific Activity

111 nmol/min/mg

Specific Lot Number

Q117-1

Purity

>90%

Concentration

0.1 $\mu\text{g}/\mu\text{l}$

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 #: I07-11G)

Active IRR (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 IRR 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 MgCl₂, 12.5mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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 (KKSREGDYMTMQIG) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active IRR, 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 IRR (Catalog #I07-11G)
 - Component 2. 5µl of 1mg/ml stock solution of substrate (Catalog #A16-58)
 - Component 3. 5µl distilled H₂O (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 H₂O.
- Step 5. Initiate the reaction by the addition of 5 µl [³³P]-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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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