

IGF1R, Active

Recombinant human protein expressed in Sf9 cells

Catalog # I02-11H

Lot # W210-1

Product Description

Recombinant human IGF1R (960-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is [NM_000875](#).

Gene Aliases

CD221, IGFR, JTK13, MGC142170, MGC142172

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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.

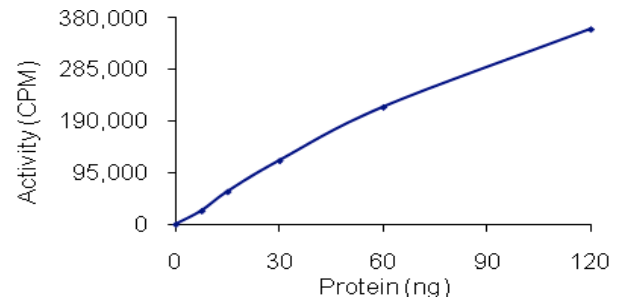
Scientific Background

IGF1R (Insulin-like Growth Factor 1) is a transmembrane tyrosine kinase receptor that is activated by IGF-1 and by the related growth factor IGF-2 (1). IGF1R mediates the effects of IGF-1 and plays an important role in growth and anabolic effects in adults. The IGF1R is implicated in several cancers, most notably breast cancer where it is highly overexpressed and functions as an anti-apoptotic agent by enhancing cell survival. In many instances, the anti-apoptotic properties of IGF1R overexpression allows cancerous cells to resist the cytotoxic properties of chemotherapeutic drugs or radiotherapy (2).

References

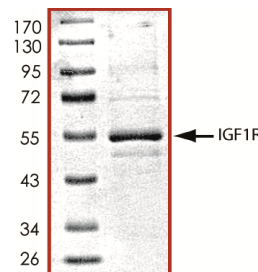
1. Yaghmaie F, et al.: "Tracking changes in hypothalamic IGF-1 sensitivity with aging and caloric restriction".. Experimental Gerontology, 2007; 42 (1-2): 148-149
2. Jones, H. et al (2004). "Insulin-like growth factor-I receptor signalling and acquired resistance to gefitinib (ZD1839; Iressa) in human breast and prostate cancer cells". Endocr. Relat. Cancer. 2004 11 (4): 793-814.

Specific Activity



The specific activity of IGF1R was determined to be **250 nmol /min/mg** as per activity assay protocol.

Purity



The purity of IGF1R was determined to be **>85%** by densitometry, approx. MW **53kDa**.

IGF1R, Active

Recombinant human protein expressed in Sf9 cells

Catalog Number	I02-11H
Specific Activity	250 nmol/min/mg
Specific Lot Number	W210-1
Purity	>85%
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 #: I02-11H)

Active IGF1R (0.1 µg/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 IGF1R 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 #: I15-58)

IGF1Rtide synthetic peptide substrate (KKKSPGEYVNIEFG) 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 IGF1R, Kinase Assay Buffer, Substrate and Enzyme 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 IGF1R (Catalog #I02-11H)
 - Component 2.** 5µl of 1mg/ml stock solution of substrate (Catalog #I15-58)
 - 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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