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Certificate of Analysis

IGF-1R (Δ 1-958), active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-465

Lot #1624808

From bulk lot# D7BN053U

Product Description: N-terminal 6His-tagged, recombinant human IGF-1R residues 959–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 48kDa.

Specific Activity (lot#1624808): 72U/mg, where one unit of IGF-1R activity is defined as 1nmol phosphate incorporated into 250 μ M (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 20 μ g of enzyme in 5.3 μ l of 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: Stable for 6 months at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

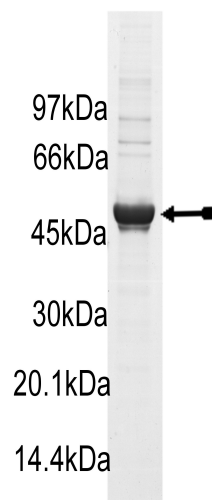
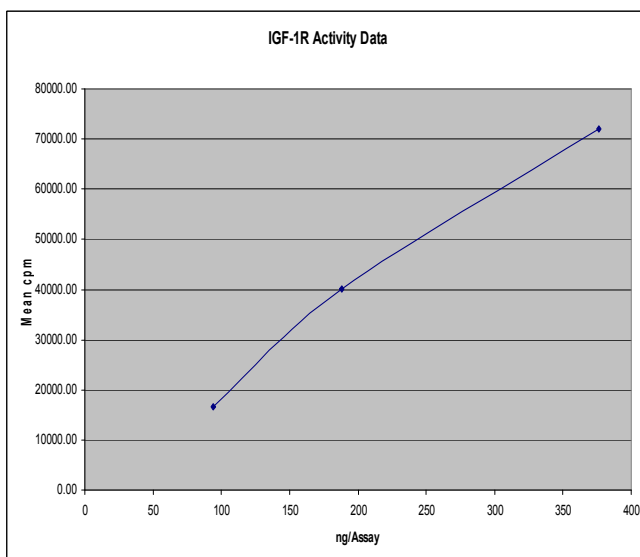
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 94–376ng of this lot of enzyme phosphorylated 250 μ M (KKKSPGEYVNIEFG) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.

MS Tryptic Fingerprint: Confirmed identity as IGF-1R with 43% amino acid coverage of the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 μ g of active IGF-1R.



Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock to reaction mixture.
3. **MnCl₂:** Use at a final assay concentration of 10mM. Prepare a 200mM stock and add 1.25µl of stock per assay point.
4. **IGF-1R, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 94–376ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5µl of 10 x reaction buffer to wells.
2. Add 2.5µl of **(KKKSPGEYVNIEFG)**.
3. Add 1.25µl of MnCl₂.
4. Add 6.25µl of dH₂O.
5. Add **2.5µl (94–376ng) IGF-1R, active**.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl 3% phosphoric acid to wells.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

IGF-1R Sequence Information

Protein	human IGF-1R
Tags	N-terminal 6His
Native sequence	H16 of the recombinant protein is equivalent to H959 of human IGF-1R.
Accession number	GenBank X04434

Recombinant IGF-1R amino acid sequence:

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1 MAHHHHHHEN LYFQGHRKRN NSRLGNGVLY ASVNPEYFSA ADVYVPDEWE VAREKITMSR
61 ELGQGSFGMV YEGVAKGVVK DEPETRVAIK TVNEAASMRE RIEFLNEASV MKEFNCHHV
121 RLLGVVSQGG PTLVIMELMT RGDLSYLR LRPENNPV LAPPSLSKMI QMAGEIADGM
181 AYLNANKFVH RDLAARNCMV AEDFTVKIGD FGMTRDIYET DYYRKGGKGL LPVRWMSPE
241 LKDGVFTTYS DVWSFGVVLW EIATLAEQPY QGLSNEQVLR FVMEGGLLDK PDNCPDMLFE
301 LMRMCWQYNP KMRPSFLEII SSIKEEMEPG FREVSFYFSE ENKLPEPEEL DLEPENMESV
361 PLDPSASSSS LPLPDRHSGH KAENGP GPV LVLRFASFDER QPYAHMNGGR KNERALPLPQ
421 SSTC
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Recombinant IGF-1R nucleotide sequence:

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1 atggcgcatc accatcacca tcatgaaaa ctgtattttc agggccatag aaagagaaat
61 aacagcaggc tggggaatgg agtgctgtat gcctctgtga acccggagta cttcagcgct
121 gctgatgtgt acgttcctga tgagtgggag gtggctcggg agaagatcac catgagccgg
181 gaacttgggc aggggtcgtt tgggatggtc tatgaaggag ttgccaaagg tgtggtgaaa
241 gatgaacctg aaaccagagt ggccattaaa acagtgaacg aggcgcgaag catgctgag
301 aggattgagt ttctcaacga agcttctgtg atgaaggagt tcaattgtca ccatgtggtg
361 cgattgctgg gtgtggtgtc ccaaggccag ccaacactgg tcatcatgga actgatgaca
421 cggggcgatc tcaaaagtta tctccggtct ctgaggccag aaatggagaa taatccagtc
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541 gcatacctca acgccaataa gttcgtccac agagaccttg ctgcccggaa ttgcatggta
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1201 cagccttacg cccacatgaa cgggggcccgc aagaacgagc gggccttgcc gctgccccag
1261 tcttcgacct gctga
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Reviewed and approved by site quality representative.

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