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## Certificate of Analysis

### IGF-1R ( $\Delta$ 1-958), active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-465

Lot # 1613803

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 Ni<sup>2+</sup>/NTA agarose. Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 48kDa.

**Specific Activity (lot# 1613803):** 72U/mg, where one unit of IGF-1R activity is defined as 1nmol phosphate incorporated into 250 $\mu$ M (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100 $\mu$ M.

**Formulation:** 20 $\mu$ g of enzyme in 5.3 $\mu$ 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.

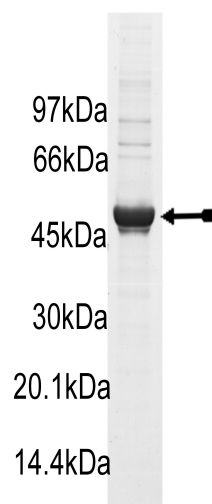
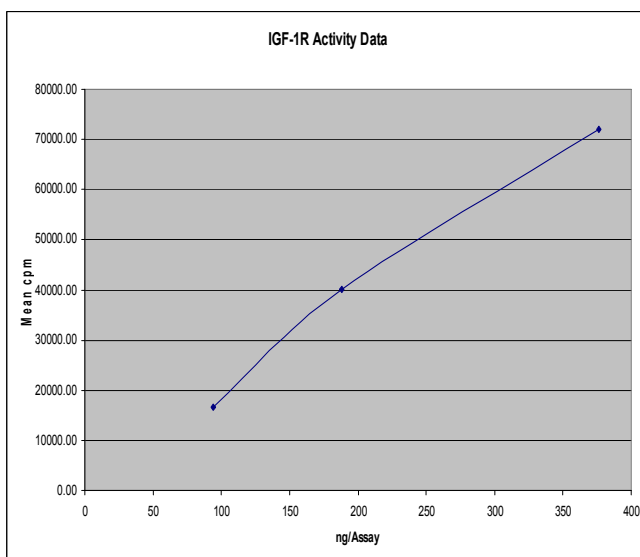
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 94–376ng of this lot of enzyme phosphorylated 250 $\mu$ 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 $\mu$ g of active IGF-1R.



## Kinase Assay Protocol

### Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na<sub>3</sub>VO<sub>4</sub>, 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. **MnCl<sub>2</sub>:** 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 Na<sub>3</sub>VO<sub>4</sub>, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 94–376ng per assay point.
5. **[γ-<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[γ-<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-<sup>33</sup>P]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 MnCl<sub>2</sub>.
4. Add 6.25μl of dH<sub>2</sub>O.
5. Add **2.5μl (94–376ng) IGF-1R, active**.
6. Add 10μl of diluted [γ-<sup>33</sup>P]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

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

### Recombinant IGF-1R amino acid sequence:

```
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 LVLRSFDER QPYAHMNGGR KNERALPLPQ
421 SSTC
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### Recombinant IGF-1R nucleotide sequence:

```
1 atggcgcatc accatcacca tcatgaaaac ctgtattttc agggccatag aaagagaaat
61 aacagcaggc tggggaatgg agtgctgtat gcctctgtga acccgagta cttcagcgct
121 gctgatgtgt acgttcctga tgagtgggag gtggctcggg agaagatcac catgagccgg
181 gaacttgggc aggggtcgtt tgggatggtc tatgaaggag ttgccagggt tgtggtgaaa
241 gatgaacctg aaaccagagt ggccattaaa acagtgaacg aggccgcaag catgctgag
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361 cgattgctgg gtgtggtgtc ccaaggccag ccaacactgg tcatcatgga actgatgaca
421 cggggcgatc tcaaaagtta tctccggtct ctgaggccag aaatggagaa taatccagtc
481 ctagcacctc caagcctgag caagatgatt cagatggccg gagagattgc agacggcatg
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901 ctgatgcgca tgtgctggca gtataacccc aagatgaggc cttccttctt ggagatcatc
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1081 cccctggacc cctcggcctc ctgcctctcc ctgccaactgc ccgacagaca ctcaggacac
1141 aaggccgaga acggccccgg ccctggggtg ctggtcctcc gcgccagctt cgacgagaga
1201 cagccttacg cccacatgaa cgggggccgc aagaacgagc gggccttgcc gctgccccag
1261 tcttcgacct gctga
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Reviewed and approved by site quality representative.

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