

Certificate of Analysis

IGF-1R, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-465, 14-465-K, 14-465M

Parent Lot # WAB0189

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

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 78% by SDS-PAGE and Coomassie blue staining. MW = 48kDa.

Specific Activity (Parent lot# WAB0189): 97U/mg, where one unit of IGF-1R, active activity is defined as 1nmol phosphate incorporated into 250μM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 2.03mg/ml of enzyme in 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.

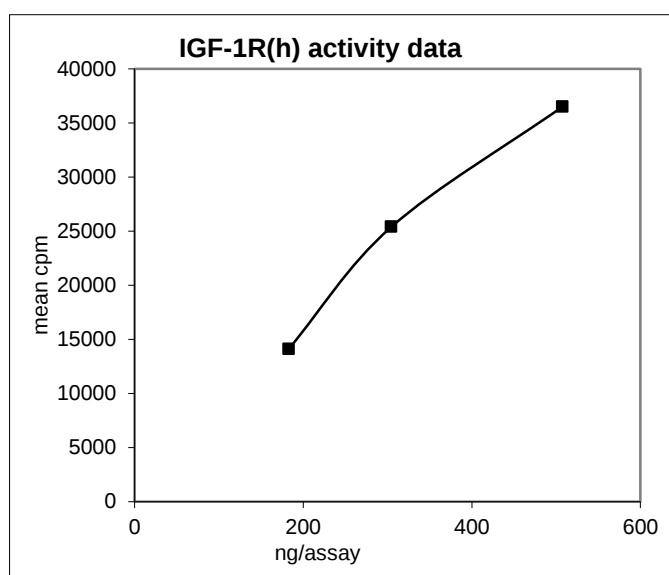
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

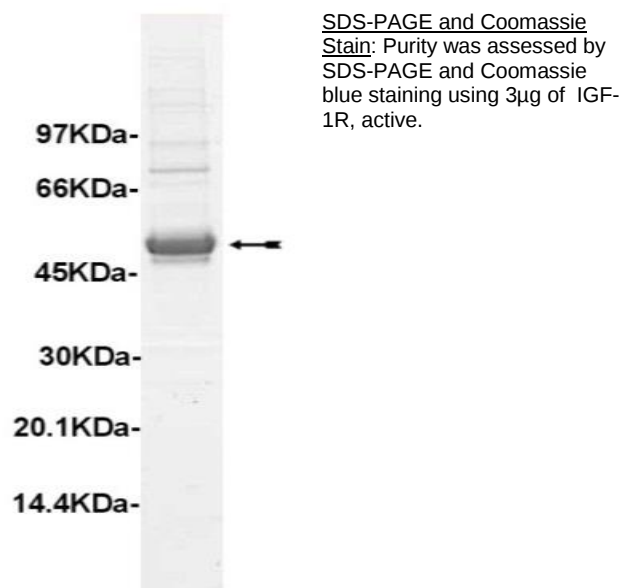
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 183–508ng of this lot of enzyme phosphorylated 250μM (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 product identity as IGF-1R with of the translated sequence listed on page three.



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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. **Manganese Chloride (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 183–508ng 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 (183–508ng) 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 NSRLNGNVLY ASVNPEYFSA ADVYVPDEWE VAREKITMSR
61 ELQGGSFGMV YEGVAKGVVK DEPTRVAIK TVNEAASMRE RIEFLNEASV MKEFNCHHV
121 RLLGVVSQGG PTLVIMELMT RGDLSYLR LRPENNNPV LAPPSSKMI QMAGEIADGM
181 AYLNANKFVH RDLAARNCMV AEDFTVKIGD FGMTRDIYET DYYRKGGKGL LPVRWMSPE
241 LKDGVTFTYS DVWSFGVVLW EIATLAEQPY QGLSNEQVLR FVMEGGLLDK PDNCPDMLFE
301 LMRMCWQYNP KMRPSFLEII SSIKEEMEPG FREVSFYFSE ENKLPEPEEL DLEPENMESV
361 PLDPSASSSS LPLPDRHSGH KAENGP GPV LVLASFDER QPYAHMNGGR KNERALPLPQ
421 SSTC

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Recombinant IGF-1R nucleotide sequence:

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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 ttgccaaggg tgtggtgaaa
241 gatgaacctg aaaccagagt ggccattaaa acagtgaacg aggccgcaag catgctgtag
301 aggattgagt tttcaacga agcttctgtg atgaaggagt tcaattgtca ccatgtggtg
361 cgattgctgg gtgtggtgtc ccaaggccag ccaacactgg tcatcatgga actgatgaca
421 cggggcgatc tcaaaagtta tctccgtct ctgaggccag aaatggagaa taatccagtc
481 ctgacacctc caagcctgag caagatgatt cagatggccg gagagattgc agacggcatg
541 gcataacctc acgccaataa gttctgccac agagaccttg ctgcccggaa ttgcatggta
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721 ctcaaggatg gagtcttcac cacttactcg gacgtctggt ccttcggggt cgtcctctgg
781 gagatcgcca cactggccga gcagccctac cagggttggt ccaacgagca agtccttcgc
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901 ctgatgcgca tgtgctggca gtataacccc aagatgaggc cttccttcct ggagatcatc
961 agcagcatca aagaggagat ggagcctggc ttccgggagg tctccttcta ctacagcgag
1021 gagaacaagc tgcccagacc ggaggagctg gacctggagc cagagaacat ggagagcgtc
1081 cccctggacc cctcgccctc ctgctcctcc ctgccactgc ccgacagaca ctcaggacac
1141 aaggccgaga acggccccgg ccctggggtg ctggctcctc gcgccagctt cgacgagaga
1201 cagccttacg ccacatgaa cgggggcccgc aagaacgagc gggccttgcc gctgccccag
1261 tcttcgacct gctga

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Reviewed and approved by site quality representative.

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