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Certificate of Analysis

IGF-1R, activated

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-802, 14-802-K, 14-802M
Parent Lot # D7MN033N

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 amino acids 959–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Autoactivated on column by incubating with Mg/ATP, excess ATP and MgAc removed by multiple column wash steps.
Purity 88.3% by SDS-PAGE and Coomassie blue staining. MW = 48kDa.

Specific Activity (Parent lot# D7MN033N): 3661U/mg, where one unit of IGF-1R activity is defined as 1nmol phosphate incorporated into 500µM IGFtide (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.984mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5mM β-glycerophosphate, 1mM Na_3VO_4. Frozen solution.

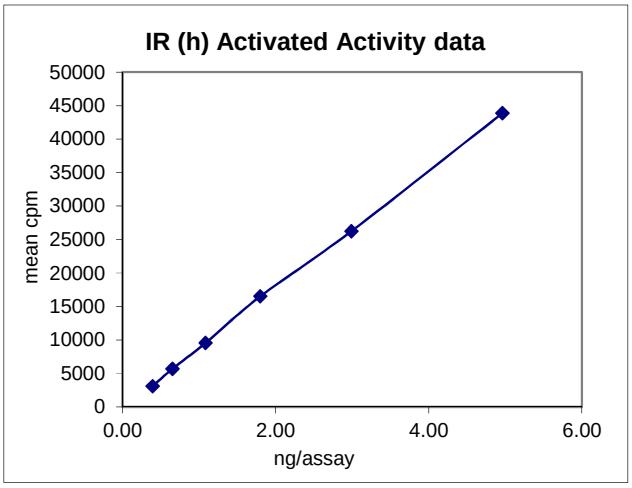
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

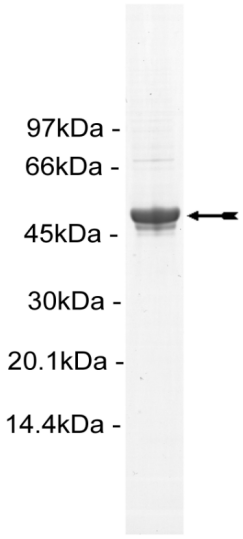
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.4–5.0ng of this lot of enzyme phosphorylated 500µM IGFtide (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 the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of IGF-1R, activated.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Na_3VO_4:** Use at a final assay concentration of 1mM. Prepare a 100mM stock and add 0.25µl of stock per assay point.
3. **Na-β-glycerophosphate:** Use at a final assay concentration of 5mM. Prepare a 1M stock and add 0.125µl of stock per assay point.
4. **IGFtide (KKKSPGEYVNIEFG):** Use at a final assay concentration of 500µM. Prepare a 5mM stock and add 2.5µl of stock per assay point.
5. **IGF-1R, activated:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–5.0ng per assay point.
6. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells
2. Add 2.5µl of **IGFtide (KKKSPGEYVNIEFG)**.
3. Add **2.5µl (0.4–5.0ng) IGF-1R, activated**.
4. Add 4.625µl of dH_2O.
5. Add 0.25µl Na_3VO_4
6. Add 0.125µl Na-β-glycerophosphate.
7. Add 10µl of diluted [γ-^{33}P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. 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.

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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 MKEFNCHHVV
 121 RLLGVVSQGQ PTLVIMELMT RGDLKSYLRS LRPEMENNPV LAPPSLSKMI QMAGEIADGM
 181 AYLNANKFVH RDLAARNCMV AEDFTVKIGD FGMTRDIYET DYYRKGGKGL LPVRWMSPES
 241 LKDGVFTTYS DVWSFGVVLW EIATLAEQPY QGLSNEQVLR FVMEGGLLDK PDNCPDMLFE
 301 LMRMCWQYNP KMRPSFLEII SSIKEEMEPG FREVSFYYSE ENKLPEPEEL DLEPENMESV
 361 PLDPSASSSS LPLPDRHSGH KAENGPGPGV LVLRASFDER 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 acccggagta cttcagcgct
 121 gctgatgtgt acgttcctga tgagtgggag gtggctcggg agaagatcac catgagccgg
 181 gaacttgggc aggggtcgtt tgggatggtc tatgaaggag ttgccaaggg tgtggtgaaa
 241 gatgaacctg aaaccagagt ggccattaaa acagtgaacg aggccgcaag catgcgtgag
 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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1081 cccctggacc cctcggcctc ctcgtcctcc ctgccactgc 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.