

Certificate of Analysis

Insulin Receptor, activated

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-803, 14-803-K, 14-803M

Parent Lot # D7MN052N

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 Insulin Receptor amino acids 1005-1310, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Autoactivated on column by incubating with Mg/ATP, excess ATP and MgAc removed by multiple column wash steps. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 36.8kDa.

Formulation: 2.868mg/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 Na₃VO₄. 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.

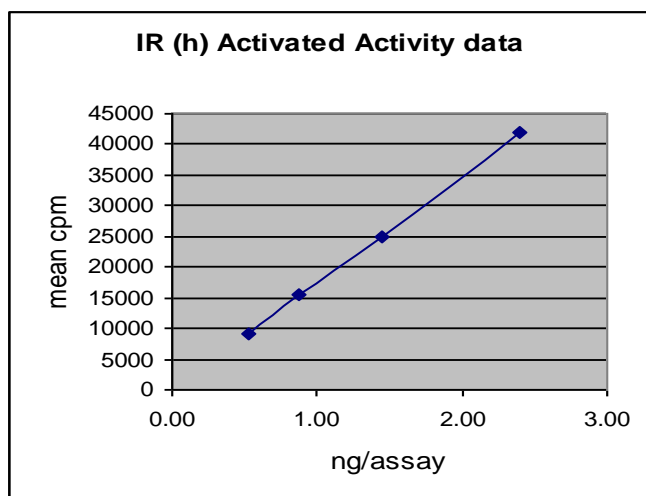
Specific Activity (Parent lot# D7MN052N): 7272U/mg, where one unit of IR activity is defined as 1nmol phosphate incorporated into 500μM IGFtide (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100μM.

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: 0.52–2.39ng 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 IR with the translated sequence listed on page three.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Na₃VO₄:** 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 1 M stock and add 0.125 µl 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. **IR, activated:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.52–2.39ng per assay point.
6. **[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 5µl of 5 x reaction buffer per assay to wells
2. Add 2µl of IGFtide (**KKKSPGEYVNIEFG**).
3. Add **2.5µl (0.52–2.39ng) IR, activated**.
4. Add 4.625µl of dH₂O.
5. Add 0.25µl Na₃VO₄
6. Add 0.125µl Na-β-glycerophosphate.
7. Add 10µl of diluted [γ-³³P]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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Insulin Receptor Sequence Information

<u>Protein</u>	Human Insulin Receptor
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	V16 of the recombinant protein is equivalent to V1005 of human Insulin Receptor
<u>Accession number</u>	GenBank NM_000208

Recombinant Insulin Receptor amino acid sequence:

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1 MAHHHHHHHEN LYFQGVFPCS VYVPDEWEVS REKITLLREL GQGSFGMVYE GNARDIIKGE
61 AETRVAVKTV NESASLRERI EFLNEASVMK GFTCHHVRL LGVVSKEGPT LVVMELMAHG
121 DLKSYLRSLR PEAENNPGRP PPTLQEMIOM AAEIADGMAY LNAKKFVHRD LAARNCMVAH
181 DFTVKIGDFG MTRDIYETDY YRKGKGGLLP VRWMAPESLK DGVFTTSSDM WSFGVVLWEI
241 TSLAEQPYQG LSNEQVLKFV MDGGYLDQPD NCPERVTDLM RMCWQFNPKM RPTFLEIVNL
301 LKDDLHPSFP EVSFFHSEEN K

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Recombinant Insulin Receptor nucleotide sequence:

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1 atggcgcac accatcacca tcatgaaaac ctgtattttc agggcgtggt tccatgctct
61 gtgtacgtgc cggacgagtg ggaggtgtct cgagagaaga tcaccctcct tcgagagctg
121 gggcagggct ccttcggcat ggtgtatgag ggcaatgcca gggacatcat caagggtag
181 gcagagaccc gcgtggcggg gaagacggtc aacgagtcag ccagtctccg agagcggatt
241 gagttcctca atgaggcctc ggtcatgaag ggcttcacct gccatcacgt ggtgcgcctc
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421 ccccttacct ttcaagagat gattcagatg gcggcagaga ttgctgacgg gatggcctac
481 ctgaacgcc aagaagttgt gcatcgggac ctggcagcga gaaactgcat ggtcgcccat
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961 aagtaa

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