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Certificate of Analysis

Insulin Receptor, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-466, 14-466-K, 14-466M
Parent Lot # WAE0358

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, residues 1005–1310, expressed by baculovirus in SF21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 92% by SDS-PAGE and Coomassie blue staining. MW = 36.8kDa.

Specific Activity (Parent lot# WAE0358): 42U/mg, where one unit of Insulin Receptor, active activity is defined as 1nmol phosphate incorporated into 250µM (KKSRGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.92mg/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. Frozen solution.

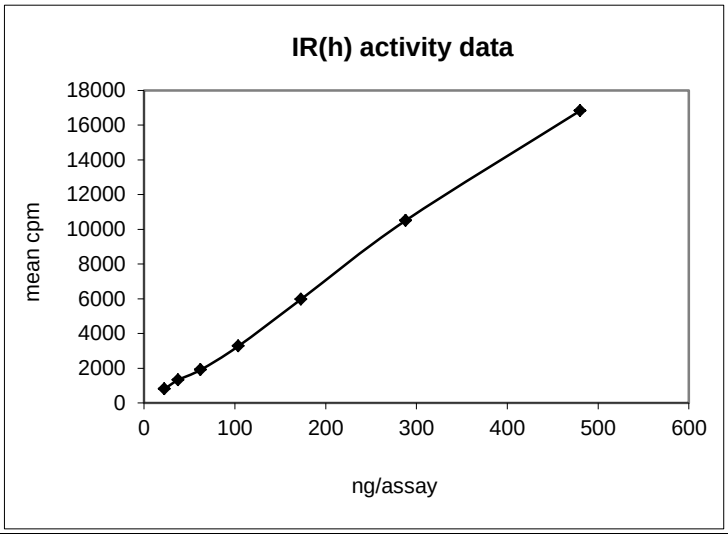
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

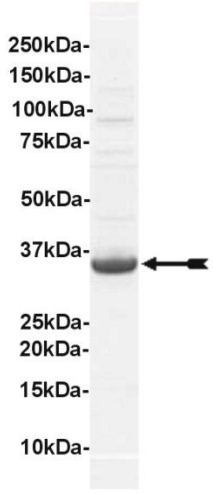
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 22.39–480ng of this lot of enzyme phosphorylated 250µM (KKSRGDYMTMQIG) 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 Insulin receptor with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Insulin Receptor, active.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol.
2. **Manganese Chloride:** Use at a final assay concentration of 10mM. Prepare a 200mM stock. Add 1.25µl of stock per assay point.
3. **(KKSRGDYMTMQIG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock. Add 2.5µl of stock per assay point
4. **Insulin Receptor, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 22.39–480ng per assay point.
5. **[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 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 1.25µl of $MnCl_2$.
3. Add 2.5µl of substrate peptide **(KKSRGDYMTMQIG)**.
4. Add **2.5µl (22.39–480ng) insulin receptor, active**.
5. Add 6.25µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
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.

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Insulin Receptor Sequence Information

Protein human Insulin Receptor

Tags *N*-terminal 6His

Native sequence V16 of the recombinant protein is equivalent to V1005 of human Insulin Receptor

Accession number GenBank NM_000208

Recombinant human Insulin Receptor amino acid sequence:

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  1 MAHHHHHHEN LYFQGVFPCS VYVPDEWEVS REKITLLREL GQGSFGMVYE GNARDIIKGE
 61 AETRVAVKTV NESASLRERI EFLNEASVMK GFTCHHVVRL LGVVSKGQPT LVVMELMAHG
121 DLKSYLRSLR PEAENNPGRP PPTLQEMIQM AAEIADGMAY LNAKKFVHRD LAARNCMVAH
181 DFTVKIGDFG MTRDIYETDY YRKGGKGLLP VRWMAPESLK DGVFTTSSDM WSFGVVLWEI
241 TSLAEQPYQG LSNEQVLKFV MDGGYLDQPD NCPERVTDLM RMCWQFNPKM RPTFLEIVNL
301 LKDDLHPSFP EVSFFHSEEN K
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Recombinant human Insulin Receptor nucleotide sequence:

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  1 atggcgcatc accatcacca tcatgaaaac ctgtattttc agggcgtgtt tccatgctct
 61 gtgtacgtgc cggacgagtg ggaggtgtct cgagagaaga tcaccctcct tcgagagctg
121 gggcagggct ccttcggcat ggtgtatgag ggcaatgcca gggacatcat caagggtgag
181 gcagagaccc gcgtggcggt gaagacggtc aacgagtcag ccagtctccg agagcggatt
241 gagttcctca atgaggcctc ggtcatgaag ggcttcacct gccatcacgt ggtgcgcctc
301 ctgggagtgg tgtccaaggg ccagcccacg ctggtggtga tggagctgat ggctcacgga
361 gacctgaaga gctacctccg ttctctgcgg ccagaggctg agaataatcc tggccgccct
421 ccccctaccc ttcaagagat gattcagatg gcggcagaga ttgctgacgg gatggcctac
481 ctgaacgcca agaagtttgt gcatcgggac ctggcagcga gaaactgcat ggtcgcccat
541 gattttactg tcaaaattgg agactttgga atgaccagag acatctatga aacggattac
601 taccggaaag ggggcaaggg tctgctccct gtacggtgga tggcaccgga gtccctgaag
661 gatggggtct tcaccacttc ttctgacatg tggtcctttg gcgtggtcct ttgggaaatc
721 accagcttgg cagaacagcc ttaccaaggc ctgtctaatg aacaggtgtt gaaatttgtc
781 atggatggag ggtatctgga tcaacccgac aactgtccag agagagtcac tgacctcatg
841 cgcatgtgct ggcaattcaa ccccaagatg aggccaacct tcctggagat tgtcaacctg
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961 aagtaa
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Reviewed and approved by site quality representative.