



Fischer Building, Gemini Crescent Dundee,
DD2 1SW, UK
T: +44(0) 1382 561600
F: +44(0) 1382 561601
Technical Support: (US/Canada)
T: 800 437-7500 • F: 800 437-7502
www.millipore.com

Certificate of Analysis

Insulin Receptor, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-466

Lot # 1617152 From bulk lot # 33286U

Product Description: N-terminal 6His-tagged, recombinant, human Insulin Receptor, residues 1005–1310, expressed by baculovirus in SF21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 36.8kDa.

Specific Activity (lot# 1617152): 52U/mg, where one unit of Insulin Receptor activity is defined as 1nmol phosphate incorporated into 250µM (KKSRGDYMTMQIG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 10µg of enzyme in 4.3µl of 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.

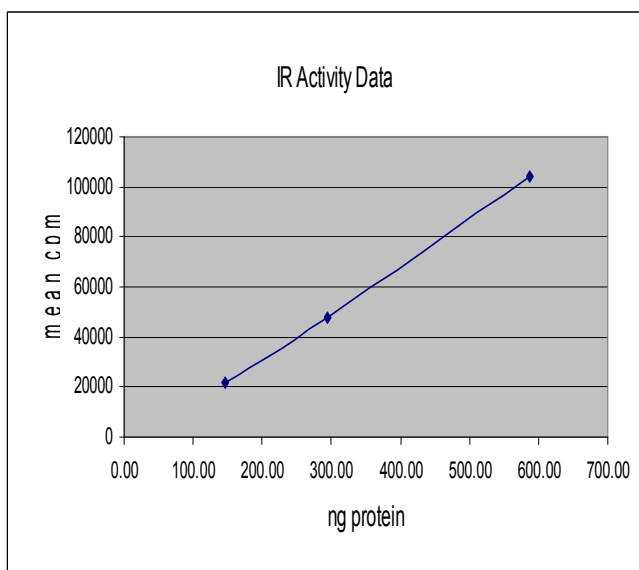
Storage and Stability: Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

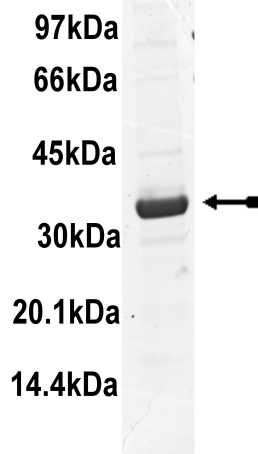
Quality Control Testing

Kinase Assay: 147–588ng 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 48% amino acid coverage of 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.



Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 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. **(KKS_RGDYMTMQIG):** 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 Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 147–588ng 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 per assay to wells.
2. Add 1.25µl of MnCl₂.
3. Add 2.5µl of substrate peptide **(KKS_RGDYMTMQIG)**.
4. Add **2.5µl (147–588ng) insulin receptor, active**.
5. Add 6.25µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]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.

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 human Insulin Receptor amino acid sequence:

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1 MAHHHHHHEN LYFQGVFPCS VYVPDEWEVS REKITLLREL GQGSFGMVYE GNARDIIKGE
61 AETRVAVKTV NESASLRERI EFLNEASVMK GFTCHHVVRL LGVVSQKQPT LVVMELMAHG
121 DLKSYLRSLR PEAENNPGRP PPTLQEMIQM AAEIADGMAY LNAKKFVHRD LAARNCMVAH
181 DFTVKIGDFG MTRDIYETDY YRKGKGLLP VRWMAPESLK DGVFTTSSDM WSFGVVLWEI
241 TSLAEQPYQG LSNEQVLKQV 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 agggcgtggt tccatgctct
61 gtgtacgtgc cggacgagtg ggaggtgtct cgagagaaga tcaccctcct tccagagctg
121 gggcagggct ctttcggcat ggtgatgag ggcaatgcca gggacatcat caagggtag
181 gcagagacc gcgtggcggg gaagacggtc aacgagtcag ccagtctccg agagcggatt
241 gagttcctca atgaggcctc ggtcatgaag ggcttcacct gccatcacgt ggtgcgcctc
301 ctgggagtg gtcacaagg ccagcccacg ctggtggtga tggagctgat ggctcacgga
361 gacctgaaga gctacctccg ttctctgcgg ccagaggctg agaataatcc tggccgccct
421 cccctaccc ttcaagagat gattcagatg gcggcagaga ttgctgacgg gatggcctac
481 ctgaacgcca agaagtttgt gcatcgggac ctggcagcga gaaactgcat ggtcgcccat
541 gattttactg tcaaaattgg agactttgga atgaccagag acatctatga aacggattac
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841 cgcattgtgt ggcaattcaa cccaagatg aggccaacct tcctggagat tgtcaacctg
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961 aagtaa
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Reviewed and approved by site quality representative.

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