

Certificate of Analysis

EGFR, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-531, 14-531-K, 14-531M

Parent Lot # WAB0528

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 GST-tagged human EGFR kinase domain residues 696–end. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione-agarose. Purity 60% by SDS-PAGE and Coomassie blue staining. MW = 86kDa.

Specific Activity (Parent lot# WAB0528): 126U/mg, where one unit of EGFR, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.36mg/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.

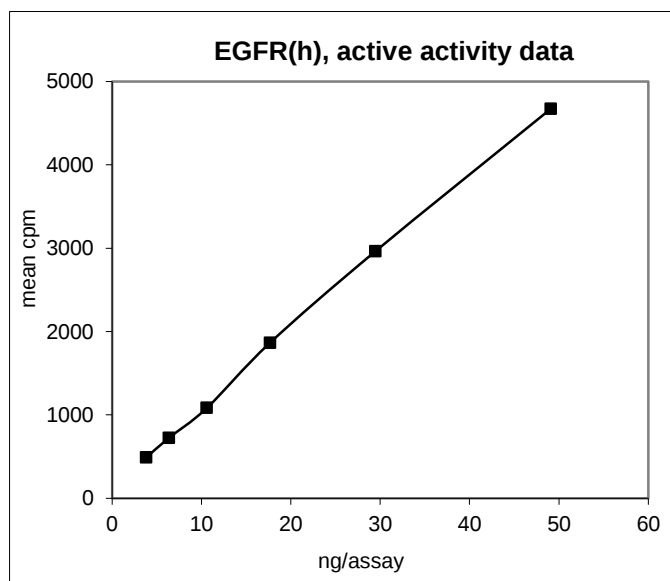
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

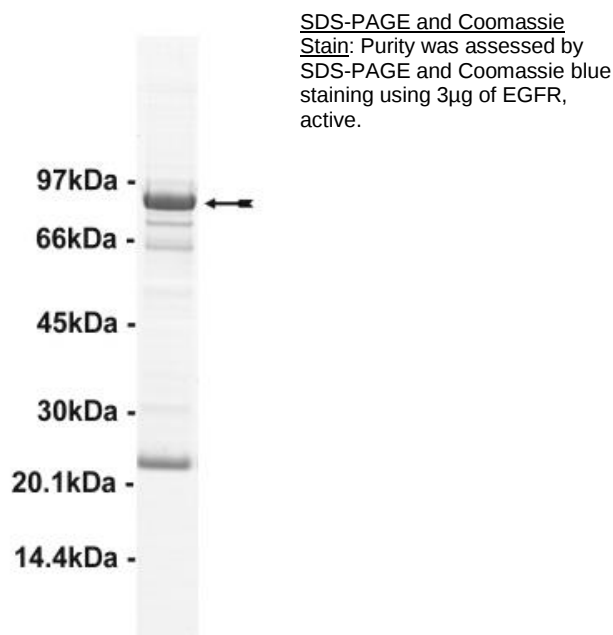
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–49ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) 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 EGFR with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Manganese Chloride (MnCl₂):** Use at a final assay concentration of 10mM. Prepare a 100mM stock and add 2.5µl of stock per assay point.
- 3. Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1 mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
- 4. EGFR, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–49ng 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:

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add 2.5µl of manganese chloride.
4. Add 2.5µl of dH₂O.
5. Add **2.5µl (4–49ng) EGFR, active**.
6. Add 10µl of diluted [γ-³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Transfer a 20µl aliquot onto the centre of a 2cm x 2cm **P81** paper square.
9. Wash the assay squares twice for 5 minutes with 75mM phosphoric acid.
10. Wash the assay squares once for 2 minutes with acetone.
11. Transfer the assay squares to a scintillation vial and add 1ml of scintillation cocktail.
12. 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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EGFR Sequence Information

<u>Protein</u>	Human EGFR
<u>Tags</u>	N-Terminal GST
<u>Native sequence</u>	G241 of the recombinant protein is equivalent to G696 of human EGFR
<u>Accession number</u>	GenBank X00588

Recombinant EGFR amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSMa IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFGQPEF KGMGIRNSKG
241 GEAPNQALLR ILKETEFKKI KVLGSGAFGT VYKGLWIPEG EKVKIPVAIK ELREATSPKA
301 NKEILDEAYV MASVDNPHVC RLLGICLTST VQLITQLMPF GCLLDYVREH KDNIGSQYLL
361 NWCVQIAKGM NYLEDRLRVH RDLAARNVLV KTPQHVKITD FGLAKLLGAE EKEYHAEGGK
421 VPIKWMALES ILHRIYTHQS DVWSYGVTVW ELMTFGSKPY DGIPASEISS ILEKGERLPQ
481 PPICTIDVYM IMVKCWMIDA DSRPKFRELI IEFKSMARDP QRYLVIQGDE RMHLPSPDTS
541 NFYRALMDEE DMDDVVDAD EYLIPQQGFFS SPSTSRTPLL SLSATSNNNS TVACIDRNGL
601 QSCPIKEDSF LQRYSSDPTG ALTEDSIDDT FLPVPEYINQ SVPKRPAGSV QNPVYHNQPL
661 NPAPSRDPHY QDPHSTAVGN PEYLVNTVQPT CVNSTFDSPA HWAQKGSHQI SLDNPDYQQD
721 FFPKEAKPNG IFKGSTAENA EYLRVAPQSS EFIGA

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Recombinant EGFR nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccacac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtgtga attgggtttg gagtttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
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301 gatattagat acggtgtttc gagaattgca tatagtaaaag actttgaaac tctcaaagtt
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1381 gacggaatcc ctgccagcga gatctcctcc atcctggaga aaggagaacg cctccctcag
1441 ccaccatat gtaccatcga tgtctacatg atcatggtca agtgctggat gatagacgca
1501 gatagtcgcc caaagttcgc tgagttgatc atcgaattct ccaaaatggc ccgagacccc
1561 cagcgctacc ttgtcattca gggggatgaa agaatgcatt tgccaagtcc tacagactcc

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1621 aacttctacc gtgccctgat ggatgaagaa gacatggacg acgtgggtgga tgccgacgag
1681 tacctcatcc cacagcaggg cttcttcagc agcccctcca cgtcacggac tcccctcctg
1741 agctctctga gtgcaaccag caacaattcc accgtggctt gcattgatag aaatgggctg
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1921 tccgttccca aaaggccgc tggctctgtg cagaatcctg tctatcacia tcagcctctg
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2041 cccgagtatc tcaacactgt ccagcccacc tgtgtcaaca gcacattcga cagccctgcc
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2161 ttctttccca aggaagccaa gccaaatggc atctttaagg gctccacagc tgaaaatgca
2221 gaatacctaa gggtcgcgcc acaaagcagt gaatttattg gagcatga
  
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