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Certificate of Analysis

EGFR (L858R), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-626, 14-626-K, 14-626M
Parent Lot # 31001U

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, recombinant human EGFR, amino acids 696–end containing the mutation L858R. This mutation has been associated with non-small cell lung cancer patients who demonstrate clinical responsiveness to the tyrosine kinase inhibitor gefitinib (Iressa, ZD1839). Expressed by baculovirus in Sf21 insect cells. Purified using glutathione-agarose. Purity 73% by SDS-PAGE and Coomassie blue staining. MW = 85.8kDa.

Specific Activity (Parent lot# 31001U): 376U/mg, where one unit of EGFR 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: 2.2mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

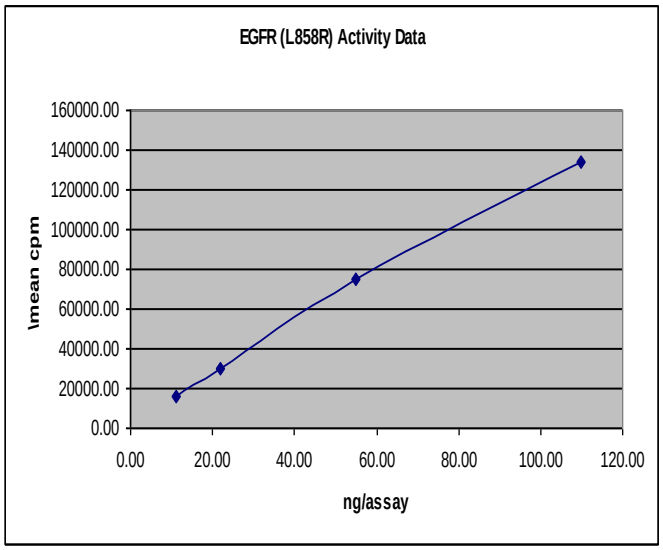
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

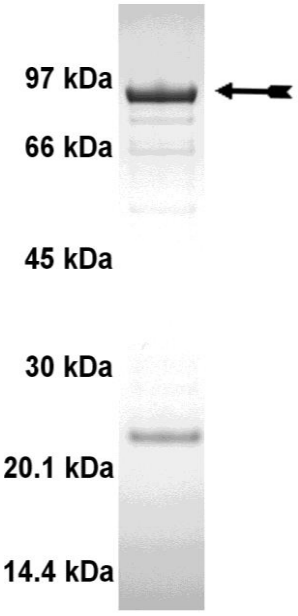
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 11–110ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu,Tyr) (4:1) 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 EGFR with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of EGFR, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Add 2.5µl of stock per assay point.
3. **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 11–110ng per assay point.
4. **[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 10 x reaction buffer per assay to wells
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (11–110ng) EGFR, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml 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 (L858R) Sequence Information

Protein Human EGFR (L858R)

Tags *N*-Terminal GST

Native sequence G241 of recombinant sequence is equivalent to G696 of native human EGFR

Accession number 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 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGMGIRNSKG
241 GEAPNQALLR ILKETEFKKI KVLGSGAFGT VYKGLWIPEG EKVKIPVAIK ELREATSPKA
301 NKEILDEAYV MASVDNPHVC RLLGICLTST VQLITQLMPF GCLLDYVREH KDNIGSQYLL
361 NWCVQIAKGM NYLEDRRLVH RDLAARNVLV KTPQHVKITD FGRAKLLGAE EKEYHAEGGK
421 VPIKWMALES ILHRIYTHQS DVWSYGVTVW ELMTFGSKPY DGIPASEISS ILEKGERLPQ
481 PPICTIDVYM IMVKCWMIDA DSRPKFRELI IEFSKMARDP QRYLVIQGDE RMHLPSPTDS
541 NFYRALMDEE DMDDVVDADE YLIPQQGFFS SPSTSRTPLL SSLSATSNNS TVACIDRNGL
601 QSCPIKEDSF LQRYSSDPTG ALTEDSIDDT FLPVPEYINQ SVPKRPAGSV QNPVYHNQPL
661 NPAPSRDPHY QDPHSTAVGN PEYLNTVQPT 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 tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
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 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
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 661 ctggaagttc tgttccaggg gcccgaattc aaaggcatgg ggatccggaa ttcaaagggt
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Reviewed and approved by site quality representative.