

Certificate of Analysis

EGFR (L861Q), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-627, 14-627-K, 14-627M

Parent Lot # 31355U

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 L861Q. 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 79.5% by SDS-PAGE and Coomassie blue staining. MW = 85.8kDa.

Specific Activity (Parent lot# 31355U): 44U/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: 2.252mg/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.

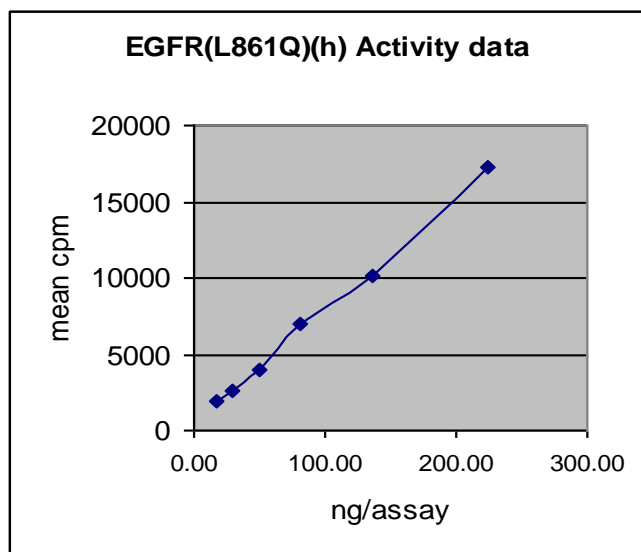
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

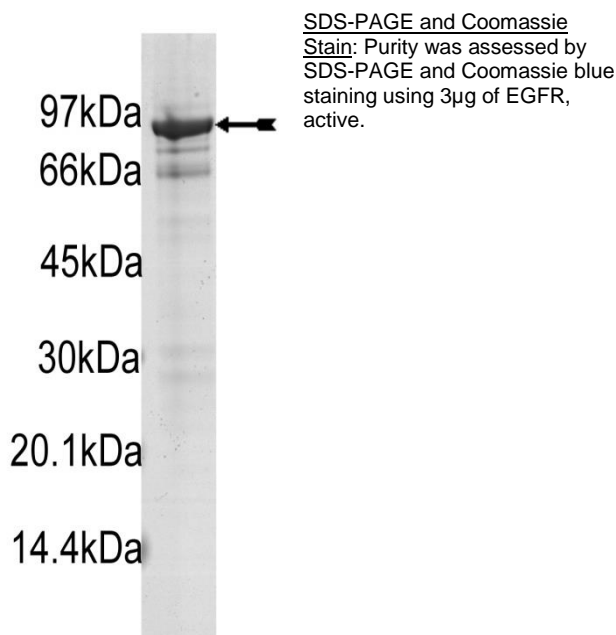
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 17.9–225ng 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.



Certificate of Analysis

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 17.9–225ng per assay point.
4. **[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 10 x reaction buffer per assay to wells
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (17.9–225ng) EGFR, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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 (L861Q) Sequence Information

<u>Protein</u>	Human EGFR (L861Q)
<u>Tags</u>	N-Terminal GST
<u>Native sequence</u>	G241 of recombinant sequence is equivalent to G696 of native human EGFR
<u>Accession number</u>	GenBank X00588

Recombinant EGF Receptor amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSM  IIRYIADKH  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCH  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LEVLFQGP  EFKMGIRNSKG
241 GEAPNQALLR  ILKETEFKKI  KVLGSGAFGT  VYKGLWIPEG  EKVKIPVAIK  ELREATSPKA
301 NKEILDEAYV  MASVDNPHVC  RLLGICLTST  VQLITQLMPF  GCLLDYVREH  KDNIGSQYLL
361 NWCVQIAKGM  NYLEDRLVH  RDLAARNVLV  KTPQHVKITD  FGLAKQLGAE  EKEYHAEGGK
421 VPIKWMALES  ILHRIYTHQS  DVWSYGVTW  ELMTFGSKPY  DGIPASEISS  ILEKGERLPQ
481 PPICTIDVYM  IMVKCWMIDA  DSRPKFRELI  IEFKSMARDP  QRYLVIQGDE  RMHLPSPTDS
541 NFYRALMDEE  DMDDVDADE  YLIPQQGFFS  SPSTSRTPLL  SLSATSNNNS  TVACIDRNL
601 QSCPIKEDSF  LQRYSSDPTG  ALTEDSIDDT  FLPVPEYINQ  SVPKRPAGSV  QNPVYHNQPL
661 NPAPSRDPHY  QDPHSTAVGN  PEYLNTVQPT  CVNSTFDSPA  HWAQKGSQI  SLDNPDYQQD
721 FFPKEAKPNG  IFKGSTAENA  EYLRVAPQSS  EFIGA

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Recombinant EGF Receptor nucleotide sequence:

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Certificate of Analysis

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1861 gccttgactg aggacagcat agacgacacc ttctctccag tgcctgaata cataaaccag
1921 tccgttccca aaaggcccgc tggtctctgt cagaatcctg tctatcacia tcagcctctg
1981 aaccccgcgc ccagcagaga cccacactac caggaccccc acagcactgc agtgggcaac
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2221 gaatacctaa gggtcgcgcc acaaagcagt gaatttattg gagcatga
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