

Certificate of Analysis

EGFR (T790M), active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-725, 14-725-K, 14-725M

Parent Lot # 1654891

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 EGF Receptor, amino acids 696–end containing the mutation T790M, expressed by baculovirus in Sf21 insect cells. Purified using glutathione-agarose. It has been shown that the T790M mutation in EGFR leads to high-level functional resistance to Iressa®. (Kobayashi et al, (2005), Cancer Res. 65, 7096-7101). Purity 85.2% by SDS-PAGE and Coomassie blue staining. MW = 86kDa.

Specific Activity (Parent lot# 1654891): 285U/mg, where one unit of EGFR (T790M) activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.379mg/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.

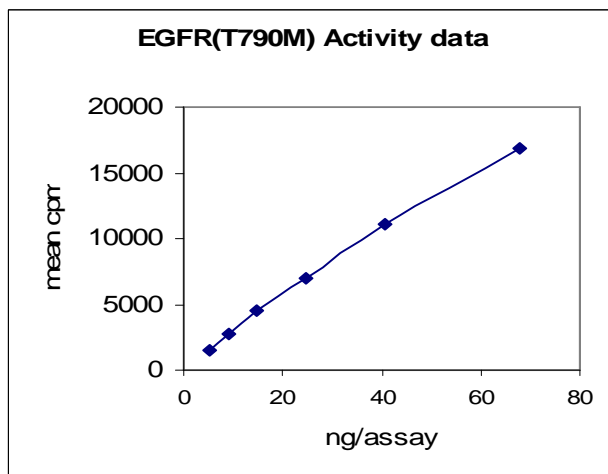
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

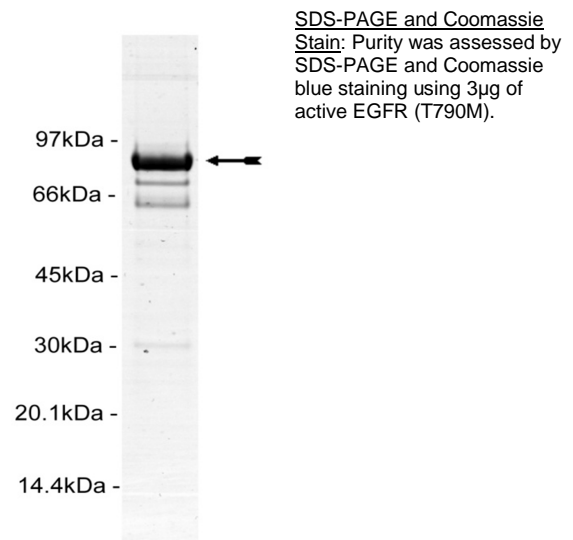
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5.4–67.7ng of this lot of enzyme phosphorylated 250µM (GGMEDIYFEFMGGKKK) 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 (T790M) with the translated native 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. **(GGMEDIYFEFMGGKKK):** Use at a final assay concentration of 250µM. Make up a 2.5mM stock. Add 2.5µl of stock per assay point.
3. **EGF Receptor (T790M), active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5.4–67.7ng 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:

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5µl (5.4–67.7ng), EGFR (T790M) 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 **P30 Filtermat**.
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 (T790M) Sequence Information

Protein	Human EGFR (T790M)
Tags	N-terminal GST
Native sequence	G241 of recombinant sequence is equivalent to G696 of human EGFR (T790M)
Accession number	GenBank X00588

Recombinant EGFR (T790M) 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 KRIEAIPOID KYLKSSKYIA WPLQGQWQATF GGDHPPKSD LEVLFQGPPEF KGMGIRNSKG
241 GEAPNQALLR ILKETEFKKI KVLGSGAFGT VYKGLWIPEG EKVKIPVAIK ELREATSPKA
301 NKEILDEAYV MASVDNPHVC RLLGICLTST VQLIMQLMPF GCLLDYVREH KDNIGSQYLL
361 NWCVQIAKGM NYLEDRLVH RDLAARNVLV KTPQHVKITD FGLAKLLGAE EKEYHAEGGK
421 VPIKWMALES ILHRIYTHQS DVWSYGVTVW ELMTFGSKPY DGIPASEISS ILEKGERLPQ
481 PPICTIDVYM IMVKCWMIDA DSRPKFRELI IEFKSMARDP QRYLVIQGDE RMHLPSPTDS
541 NFYRALMDEE DMDDVVDAD EYLIPQQGFFS SPSTSRTPLL SLSATSNNNS TVACIDRNGL
601 QSCPIKEDSF LQRYSSDPTG ALTEDSIDDT FLPVPEYINQ SVPKRPAGSV QNPVYHNQPL
661 NPAPSRDPHY QDPHSTAVGN PEYLNVTQPT CVNSTFDSPA HWAQKGSQHI SLDNPDYQQD
721 FFPKEAKPNG IFKGSTAENA EYLRVAPQSS EFIGA

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Recombinant EGFR (T790M) nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aaggcccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaaca aaaagttaga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
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421 acatatttaa atggtgatca tgaacccat cctgacttca tgttgatga cgctcttgat
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541 aaacgtattg aagctatccc acaaattgat aagtaactga aatccagcaa gtatatagca
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1381 gacggaatcc ctgccagcga gatctcctcc atcctggaga aaggagaacg cctccctcag
1441 ccaccatat gtaccatcga tgtctacatg atcatggtca agtgctggat gatagacgca
1501 gatagtcgcc caaagttccg tgagttgatc atcgaattct ccaaatggc ccgagacccc

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1561 cagcgctacc ttgtcattca gggggatgaa agaatgcatt tgccaagtcc tacagactcc
1621 aacttctacc gtgccctgat ggatgaagaa gacatggacg acgtggtgga tgccgacgag
1681 tacctcatcc cacagcaggg cttcttcagc agcccctcca cgtcacggac tcccctcctg
1741 agctctctga gtgcaaccag caacaattcc accgtggctt gcattgatag aaatgggctg
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1921 tccgttccca aaaggcccgc tggctctgtg cagaatcctg tctatcacia tcagcctctg
1981 aaccccgccg ccagcagaga cccacactac caggaccccc acagcactgc agtgggcaac
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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