

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), active activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIFYEFMGGKKK) 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.

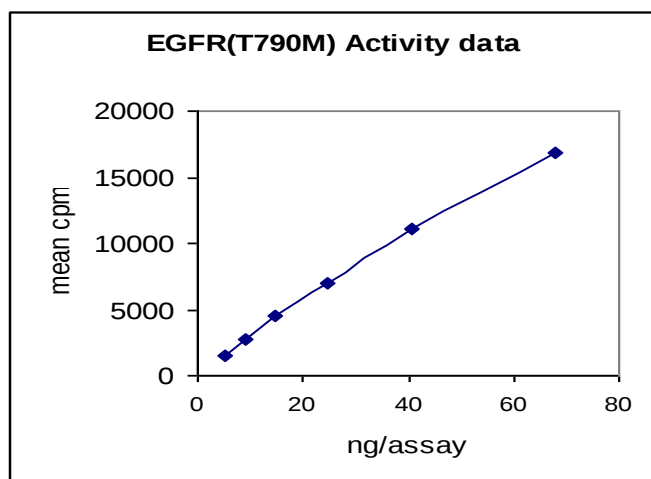
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

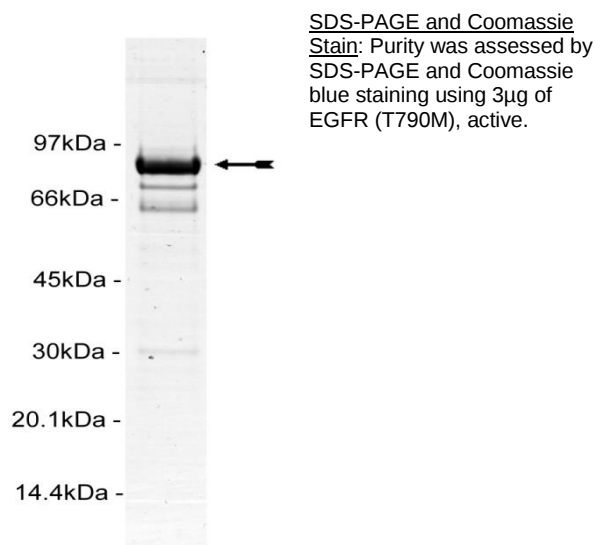
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 5.4–67.7ng of this lot of enzyme phosphorylated 250µM (GGMEDIFYEFMGGKKK) 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  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LEVLFQGPEF  KGMGIRNSKG
241  GEAPNQALLR  ILKETEFKKI  KVLGSGAFGT  VYKGLWIEG  EKVKIPVAIK  ELREATSPKA
301  NKEILDEAYV  MASVDNPHVC  RLLGICLTST  VQLIMQLMPF  GCLLDYVREH  KDNIGSQYLL
361  NWCVQIAKGM  NYLEDRLVH  RDLAARNVLV  KTPQHVKITD  FGLAKLLGAE  EKEYHAEGGK
421  VPIKWMAL  ILHRIYTHQS  DVWSYGVTVW  ELMTFGSKPY  DGIPASEISS  ILEKGERLPQ
481  PPICTIDVYM  IMVKCWMIDA  DSRPKFRELI  IEFKSMARDP  QRYLVIQGDE  RMHLPSPDTS
541  NFYRALMDEE  DMDDVVDAD  YLIPQQGFFS  SPSTSRTPLL  SLSATSNNNS  TVACIDRNGL
601  QSCPIKEDSF  LQRYSSDPTG  ALTEDSIDDT  FLPVPEYINQ  SVPKRPAGSV  QNPVYHNQPL
661  NPAPSRDPHY  QDPHSTAVGN  PEYLNVTQPT  CVNSTFDSPA  HWAQKGSHQI  SLDNPDYQQD
721  FFPKEAKPNG  IFKGSTAENA  EYLRVAPQSS  EFIGA

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Recombinant EGFR (T790M) nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatt  ttgaagaaaa  atatgaagag  catttgatg  agcgcatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
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241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaa  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcggtt  atgtcataaa
421  acataatttaa  atggtgatca  tgtaacccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gatatatgca
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721  ggagaagctc  ccaaccaagc  tctcttgagg  atcttgaagg  aaactgaatt  caaaaagatc
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1021  ggctgcctcc  tggactatgt  ccgggaacac  aaagacaata  ttggctccca  gtacctgtc
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1201  tttgggctgg  ccaaactgct  ggggtgcggaa  gagaaagaat  accatgcaga  aggaggcaaa
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1441  ccaccatat  gtaccatcga  tgtctacatg  atcatggtca  agtgctggat  gatagacgca
1501  gatagtcgcc  caaagttccg  tgagttgatc  atcgaattct  ccaaaatggc  ccgagacccc
1561  cagcgctacc  ttgtcattca  gggggatgaa  agaatgcatt  tgccaagtcc  tacagactcc
1621  aacttctacc  gtgcctgat  ggatgaagaa  gacatggacg  acgtggtgga  tgccgacgag

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1681 tacctcatcc cacagcaggg cttcttcagc agccctcca cgtcacggac tcccctcctg
1741 agctctctga gtgcaaccag caacaattcc accgtggctt gcattgatag aaatgggctg
1801 caaagctgtc ccatcaagga agacagcttc ttgcagcgat acagctcaga cccacaggc
1861 gccttgactg aggacagcat agacgacacc ttcctcccag tgctgaata cataaaccag
1921 tccgttccca aaaggccgc tggctctgtg cagaatcctg tctatcaca tcagcctctg
1981 aaccccgcg ccagcagaga cccacactac caggacccc acagcactgc agtgggcaac
2041 cccgagtatc tcaacactgt ccagcccacc tgtgtcaaca gcacattcga cagccctgcc
2101 cactgggccc agaaaggcag ccaccaaatt agcctggaca accctgacta ccagcaggac
2161 ttctttccca aggaagcaa gccaaatggc atctttaagg gctccacagc tgaaaatgca
2221 gaatacctaa gggtcgcgcc acaaagcagt gaatttattg gagcatga

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