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Certificate of Analysis

EGFR (T790M,L858R), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-721, 14-721-K, 14-721M
Parent Lot # 1691467

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 mutations T790M and L858R, expressed by baculovirus in Sf21 insect cells. Purified using glutathione-agarose. Purity 81.9% by SDS-PAGE and Coomassie blue staining. MW = 85.8kDa.

Specific Activity (Parent lot# 1691467): 107U/mg, where one unit of EGFR (T790M,L858R), active activity is defined as 1nmol phosphate incorporated into 250µM (GGMEDIYFEFMGGKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.501mg/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.

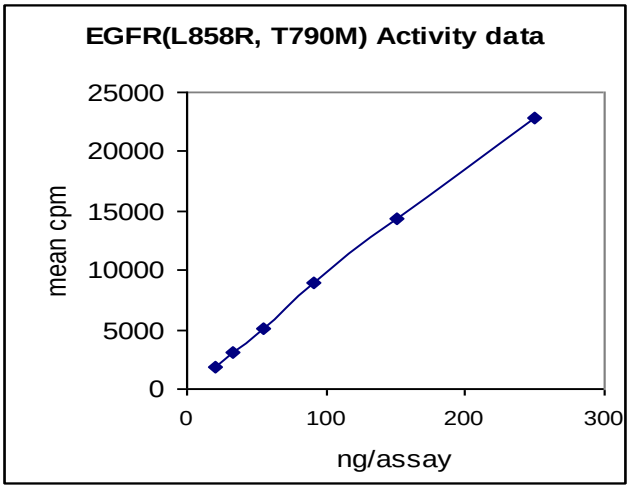
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

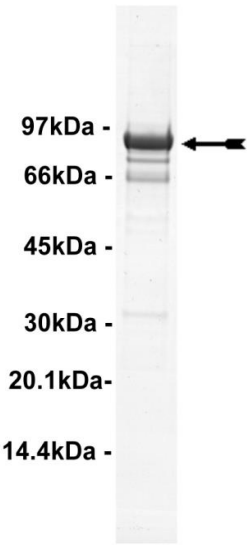
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 20–251ng 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, L858R), 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-(T790M,L858R), active.

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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. **EGFR (T790M, L858R), active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 20–251ng 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:

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(GGMEDIYFEFMGGKKK)**.
3. Add **2.5µl (20–251ng) EGFR (T790M, L858R) 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 **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-(696–end, T790M, L858R) Sequence Information

Protein Human EGFR (T790M,L858R)

Tags *N*-terminal GST

Native sequence G241 of the recombinant protein is equivalent to G696 of human EGFR (T790M,L858R). The amino acid substitution L858R is one of several heterozygous mutations that have been identified in Non-Small-Cell Lung Cancer (NSCLC) patients who have clinical responses to the EGFR inhibitor Iressa®. There is some evidence that these mutations result in elevated activity and enhanced sensitivity to Iressa® (Lynch *et al*., (2004), N. Engl. J. Med. **350**, 2129-2139).
In patients with tumours bearing Iressa®-sensitive mutations, resistant subclones containing an additional EGFR mutation, T790M, emerge in the presence of the drug (Pao *et al*., (2005), PLoS Medicine **2**, 225-235; Kwak *et al*., (2005), Proc. Natl. Acad. Sci. USA **102**, 7665-7670.). It has been shown experimentally that the T790M mutation leads to high-level functional resistance to Iressa® (Kobayashi et al., (2005), Cancer Res. 65, 7096-7101.)

Accession number GenBank X00588

Recombinant EGFR (T790M, L858R) 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 VQLIMQLMPF 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 (T790M, L858R) 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
181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgttttaaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
661 ctggaagttc tgttccaggg gcccgaattc aaaggcatgg ggatccggaa ttcaaagggt
721 ggagaagctc ccaaccaagc tctcttgagg atcttgaagg aaactgaatt caaaaagatc
781 aaagtgctgg gctccggtgc gttcggcacg gtgtataagg gactctggat cccagaaggt
841 gagaaagtta aaattcccgt cgctatcaag gaattaagag aagcaacatc tccgaaagcc
901 aacaaggaaa tcctcgatga agcctacgtg atggccagcg tggacaaccc ccacgtgtgc
961 cgcctgctgg gcatctgcct cacctccacc gtgcaactca tcatgcagct catgcccttc
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1021 ggctgcctcc tggactatgt ccgggaacac aaagacaata ttggctccca gtacctgctc
1081 aactggtgtg tgcagatcgc aaagggcatg aactacttgg aggaccgtcg cttggtgcac
1141 cgcgacctgg cagccaggaa cgtactggtg aaaacaccgc agcatgtcaa gatcacagat
1201 tttgggcggg ccaaactgct gggtgcggaa gagaaagaat accatgcaga aggaggcaaa
1261 gtgcctatca agtggatggc attggaatca attttacaca gaatctatac ccaccagagt
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1381 gacggaatcc ctgccagcga gatctcctcc atcctggaga aaggagaacg cctccctcag
1441 ccacccatat gtaccatcga tgtctacatg atcatggtca agtgctggat gatagacgca
1501 gatagtcgcc caaagttccg tgagttgatc atcgaattct ccaaaatggc ccgagacccc
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1621 aacttctacc gtgccctgat ggatgaagaa gacatggacg acgtggtgga tgccgacgag
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1741 agctctctga gtgcaaccag caacaattcc accgtggctt gcattgatag aaatgggctg
1801 caaagctgtc ccatcaagga agacagcttc ttgcagcgat acagctcaga ccccacaggc
1861 gccttgactg aggacagcat agacgacacc ttcctcccag tgcctgaata cataaaccag
1921 tccgttccca aaaggcccgc tggctctgtg cagaatcctg tctatcacaa tcagcctctg
1981 aaccccgcgc ccagcagaga cccacactac caggaccccc acagcactgc agtgggcaac
2041 cccgagtatc tcaacactgt ccagcccacc tgtgtcaaca gcacattcga cagccctgcc
2101 cactgggccc agaaaggcag ccaccaaatt agcctggaca accctgacta ccagcaggac
2161 ttctttccca aggaagccaa gccaaatggc atctttaagg gctccacagc tgaaaatgca
2221 gaatacctaa gggtcgcgcc acaaagcagt gaatttattg gagcatga
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Reviewed and approved by site quality representative.