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Certificate of Analysis

FGFR1 (V561M), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-734, 14-734-K, 14-734M
Parent Lot # D7BN045U

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 FGFR1 amino acids 456–765 containing the V561 mutation. Expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Co-ordinates encompass the protein kinase domain (478–767) according to SP P11362. The coordinates for recombinant human FGFR1 were described in Homann *et al*, J Biotech (2001); **86**:51-58.Blencke *et* al (2004) suggested mutations to a conserved threonine residue at the ATP binding site would result in inhibitor resistance. The amino acid valine 561 was mutated to a methionine in FGFR1 which corresponded to previously reported mutations found in Abl (T315) and EGFR (T766) which had been shown to confer resistance to selective inhibitors. Assay data for FGFR1 V561M had shown that this mutation had conferred resistance to PP58 (pyrido [2,3-d] pyrimidine tyrosine kinase inhibitor) compared with that of the wild type. (Blencke, S. *et al*, Chemistry & Biology (2004);**11**;691-701) Purity 100% by SDS-PAGE and Coomassie blue staining. MW = 62.3kDa.

Specific Activity (Parent lot# D7BN045U): 4741U/mg, where one unit of FGFR1 (V561M), active activity is defined as 1nmol phosphate incorporated into 500µM (GGEEEEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.2mg/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.

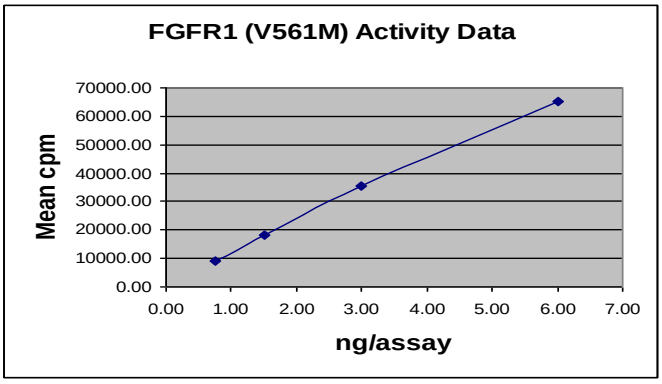
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

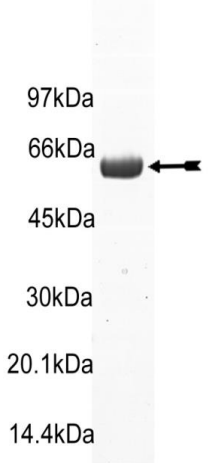
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.75–6.00ng of this lot of enzyme phosphorylated 500µM (GGEEEEYFELVKKKK) 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 product identity as FGFR1 (V561M) 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 FGFR1 (V561M), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(GGEEEEYFELVKKKK):** Use at a final assay concentration of 500µM. Prepare a 5mM stock and add 2.5µl of stock per assay point.
3. **FGFR1 (V561M), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.75–6.00ng 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 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(GGEEEEYFELVKKKK)**.
3. Add **2.5µl (0.75–6.00ng) FGFR1 (V561M), active**.
4. Add 10µl of diluted [γ-^{33}P]ATP mixture.
5. Add 5µl of dH_2O.
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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FGFR1 (V561M) Sequence Information

Protein Human FGFR1 (V561M)

Tags *N*-terminal GST

Native sequence M231 of recombinant sequence is equivalent to M456 of native human FGFR1

Accession number GenBank NM_000604

Recombinant FGFR1 (V561M) 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 MLAGVSEYEL
 241 PEDPRWELPR DRLVLGKPLG EGCFGQVVLA EAIGLDKDKP NRVTKVAVKM LKSDATEKDL
 301 SDLISEMEMM KMIGKHKNII NLLGACTQDG PLYVIMEYAS KGNLREYLQA RRPPGLEYCY
 361 NPSHNPEEQL SSKDLVSCAY QVARGMEYLA SKKCIHRDLA ARNVLVTEDN VMKIADFGLA
 421 RDIHHIDYYK KTTNGRLPVK WMAPEALFDR IYTHQSDVWS FGVLLWEIFT LGGSPYPGVP
 481 VEELFKLLKE GHRMDKPSNC TNELYMMMRD CWHAVPSQRP TFKQLVEDLD RIVALTSNQE
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Recombinant FGFR1 (V561M) 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
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 961 aacctgctgg gggcctgcac gcaggatggt cccttgtatg tcatcatgga gtatgcctcc
1021 aagggcaacc tgcgggagta cctgcaggcc cggaggcccc cagggctgga atactgctac
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1261 cgggacattc accacatcga ctactataaa aagacaacca acggccgact gcctgtgaag
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1441 gtggaggaac ttttcaagct gctgaaggag ggtcaccgca tggacaagcc cagtaactgc
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1621 taa
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Reviewed and approved by site quality representative.