

Certificate of Analysis

FGFR1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-582, 14-582-K, 14-582M

Parent Lot # 1600528

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, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 75.1% by SDS-PAGE and Coomassie blue staining. MW = 62kDa.

Specific Activity (Parent lot# 1600528): 1861U/mg, where one unit of FGFR1, active activity is defined as 1nmol phosphate incorporated into 250µM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.008mg/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.

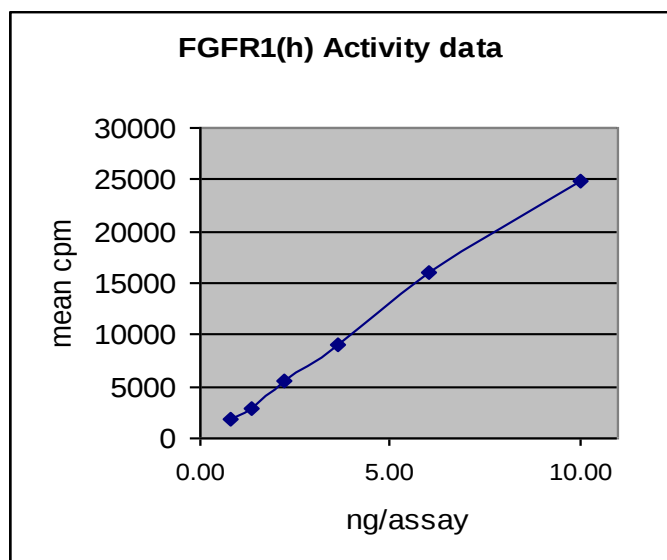
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

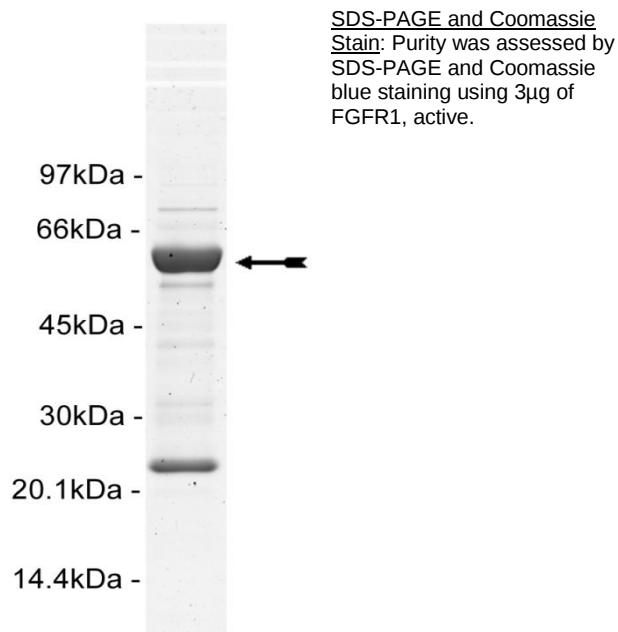
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.8–10.0ng of this lot of enzyme phosphorylated 250µM (KKKSPGEYVNIEFG) 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 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. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
3. **FGFR1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.8–10.0ng 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 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKKSPGEYVNIEFG)**.
3. Add **2.5µl (0.8–10.0ng) FGFR1, active**.
4. Add 10µl of diluted [γ -³³P]ATP mixture.
5. Add 5µl of dH₂O.
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 Sequence Information

Protein	Human FGFR1
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 amino acid sequence:

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1  MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61  GDVKLTQSM A IIRYIADKH N MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MLAGVSEYEL
241 PEDPRWELPR DRLVLGKPLG ECGFQGVVLA EAIGLDKDKP NRVTKVAVKM LKSDATEKDL
301 SDLISEMEMM KMIGKHKNI I NLLGACTQDG PLYVIVEYAS KGNLREYLQA RRPPGLEICY
361 NPSHNPEEQL SSKDLVSCAY QVARGMEYLA SKKCIHRDLA ARNVLVTE DN VMKIADFGLA
421 RDIHHIDYYK KTTNGRLPVK WMAPEALFDR IYTHQSDVWS FGVLLWEIFT LGGSPYPGVP
481 VEELFKLLKE GHRMDKPSNC TNELYMMMRD CWHAVPSQRP TFKQLVEDLD RIVALTSNQE

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Recombinant FGFR1 nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatatc ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttcca atcttcctta ttatatgtat
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241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
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1621 taa

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