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Certificate of Analysis

FGFR2, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-617, 14-617-K, 14-617M
Parent Lot # 25722U

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, 6His-tagged, recombinant, human FGFR2, amino acids 456–770, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 38.1kDa.

Specific Activity (Parent lot# 25722U): 1188U/mg, where one unit of FGFR2, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.707mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

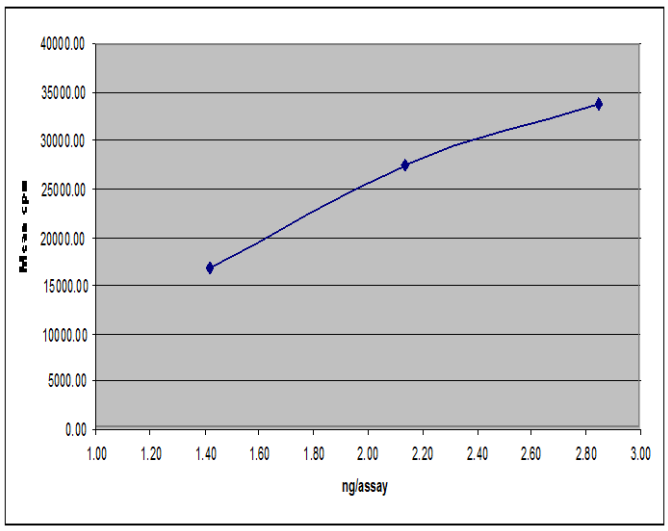
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

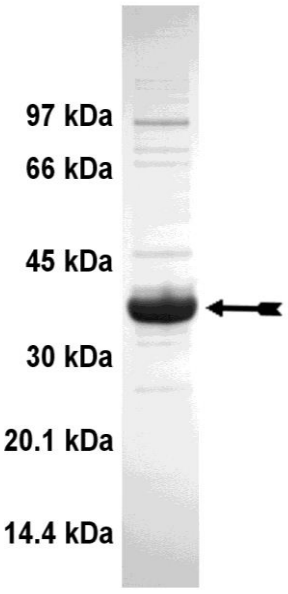
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1.42–2.85ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) 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 FGFR2 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 FGFR2, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Poly(Glu, Tyr) (4:1):** Use at a final concentration of 0.1mg/ml. Make up a 1mg/ml stock. Use 2.5µl of stock per assay point.
3. **Manganese Chloride ($MnCl_2$):** Use at a final concentration of 2.5mM. Make up a 25mM stock. Use 2.5µl of stock per assay point.
4. **FGFR2, active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.42–2.85ng per assay point.
5. **[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 **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (1.42–2.85ng) FGFR2, active**.
4. Add 2.5µl of dH_2O.
5. Add 2.5µl of 25mM $MnCl_2$
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. 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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FGFR2 Sequence Information

Protein human FGFR2

Tags *N*-terminal 6His

Native sequence D16 of the recombinant protein is equivalent to D456 of human FGFR2

Accession number GenBank NM_000141

Recombinant FGFR2 amino acid sequence:

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  1 MHHHHHHEFK GLRRQDTPML AGVSEYELPE DPKWEFPRDK LTLGKPLGEG CFGQVVMAEA
 61 VGIDKDKPKE AVTVAVKMLK DDATEKDLSD LVSEMEMMKM IGKHKNIINL LGACTQDGPL
121 YVIVEYASKG NLREYLRARR PPGMEYSYDI NRVPEEQMTF KDLVSCTYQL ARGMEYLASQ
181 KCIHRDLAAR NVLVTENNVM KIADFGLARD INNIDYYKKT TNGRLPVKWM APEALFDRVY
241 THQSDVWSFG VLMWEIFTLG GSPYPGIPVE ELFKLLKEGH RMDKPANCTN ELYMMMRDCW
301 HAVPSQRPTF KQLVEDLDRI LTLTTNEEYL
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Recombinant FGFR2 nucleotide sequence:

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  1 atgcatcatc accatcacca tgaattcaaa ggcctacgtc gacaagacac ccccatgctg
 61 gcaggggtct ccgagtatga acttccagag gacccaaaat gggagtttcc aagagataag
121 ctgacactgg gcaagcccct gggagaaggt tgctttgggc aagtggtcat ggcggaagca
181 gtgggaattg acaaagacaa gcccaaggag gcggtcaccg tggccgtgaa gatgttgaaa
241 gatgatgcca cagagaaaga cctttctgat ctggtgtcag agatggagat gatgaagatg
301 attgggaaac acaagaatat cataaatctt cttggagcct gcacacagga tgggcctctc
361 tatgtcatag ttgagtatgc ctctaaaggc aacctccgag aatacctccg agcccggagg
421 ccacccggga tggagtactc ctatgacatt aaccgtgttc ctgaggagca gatgaccttc
481 aaggacttgg tgtcatgcac ctaccagctg gccagaggca tggagtactt ggcttcccaa
541 aaatgtattc atcgagattt agcagccaga aatgttttgg taacagaaaa caatgtgatg
601 aaaatagcag actttggact cgccagagat atcaacaata tagactatta caaaaagacc
661 accaatgggc ggcttccagt caagtggatg gctccagaag ccctgtttga tagagtatac
721 actcatcaga gtgatgtctg gtccttcggg gtgttaatgt gggagatctt cactttaggg
781 ggctcgccct acccagggat tcccgtggag gaacttttta agctgctgaa ggaaggacac
841 agaatggata agccagccaa ctgcaccaac gaactgtaca tgatgatgag ggactgttgg
901 catgcagtgc cctcccagag accaacgttc aagcagttgg tagaagactt ggatcgaatt
961 ctcactctca caaccaatga ggaatacttg taa
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Reviewed and approved by site quality representative.