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Certificate of Analysis

FGFR3, active

(Recombinant enzyme expressed in SF21 insect cells)
Item # 14-464, 14-464-K, 14-464M
Parent Lot # 1832558

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 FGFR3, amino acids 447–761, expressed by baculovirus in SF21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 75.1% by SDS-PAGE and Coomassie blue staining. MW = 36.9kDa.

Specific Activity (Parent lot# 1832558): 2301U/mg, where one unit of FGFR3, 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: 0.561mg/ml in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

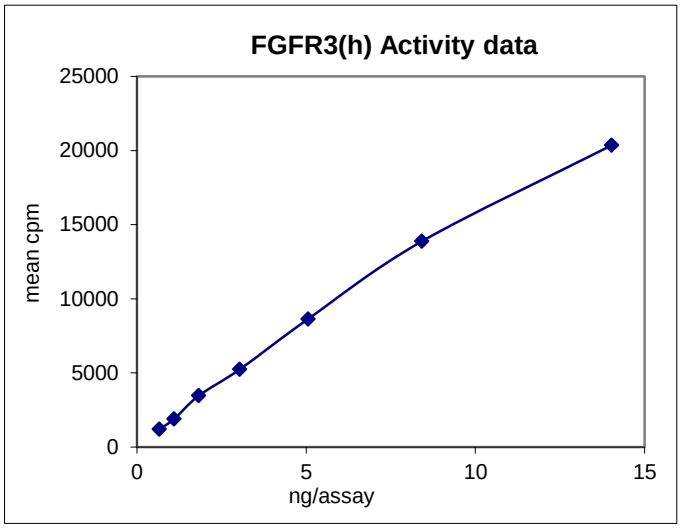
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

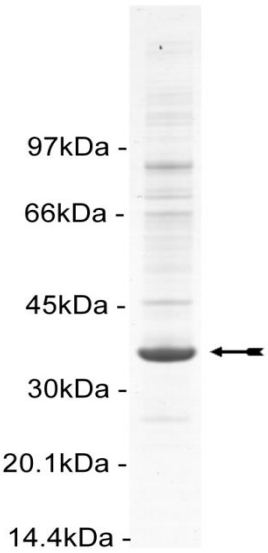
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.7–14.0ng 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 product identity as FGFR3 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of FGFR3, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Manganese Chloride ($MnCl_2$):** Use at a final assay concentration of 10mM. Prepare a 200mM stock and add 1.25µl per assay point.
3. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Make up a 1mg/ml stock. Add 2.5µl of stock per assay point.
4. **FGFR3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethnaol, 1mg/ml BSA. Use 0.7–14.0ng 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 of 1mg/ml **poly(Glu, Tyr) (4:1)**.
3. Add 1.25µl of $MnCl_2$.
4. Add 3.75µl of dH_2O.
5. Add **2.5µl (0.7–14.0ng) FGFR3, active**.
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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FGFR3 Sequence Information

Protein Human FGFR3

Tags *N*-terminal 6His

Native sequence E10 of the recombinant protein is equivalent to E447 of human FGFR3

Accession number GenBank M58051

Recombinant FGFR3 amino acid sequence:

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  1 MHHHHHHEFE GPTLANVSEL ELPADPKWEL SRARLTLGKP LGEGCFGQVV MAEAIGIDKD
 61 RAAKPVTVAV KMLKDDATDK DLSDLVSEME MMKMIGKHKN IINLLGACTQ GGPLYVLVEY
121 AAKGNLREFL RARRPPGLDY SFDTCKPPEE QLTFKDLVSC AYQVARGMEY LASQKCIHRD
181 LAARNVLVTE DNVMKIADFG LARDVHNLDY YKKTTNGRLP VKWMAPEALF DRVYTHQSDV
241 WSFGVLLWEI FTLGGSPYPG IPVEELFKLL KEGHRMDKPA NCTHDLYMIM RECWHAAPSQ
301 RPTFKQLVED LDRVLTVTST DEYL
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Recombinant FGFR3 nucleotide sequence:

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  1 atgcatcatc accatcacca tgaattcgaa ggccccacgc tggccaatgt ctccgagctc
 61 gagctgcctg ccgaccccaa atgggagctg tctcgggccc ggctgaccct gggcaagccc
121 cttggggagg gctgcttcgg ccaggtggtc atggcggagg ccatcggcat tgacaaggac
181 cgggccgcca agcctgtcac cgtagccgtg aagatgctga aagacgatgc cactgacaag
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301 atcatcaacc tgctgggcgc ctgcacgcag ggcgggcccc tgtacgtgct ggtggagtac
361 gcggccaagg gtaacctgcg ggagtttctg cgggcgcggc ggcccccggg cctggactac
421 tccttcgaca cctgcaagcc gcccgaggag cagctcacct tcaaggacct ggtgtcctgt
481 gcctaccagg tggcccgggg catggagtac ttggcctccc agaagtgcat ccacagggac
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961 gacgagtacc tgtga
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Reviewed and approved by site quality representative.