

Certificate of Analysis

FGFR2 (N549H), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-742, 14-742-K, 14-742M

Parent Lot # 1606092

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 (N549H), amino acids 456–770, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

This N549H is located within the tyrosine kinase domain and has been found in patients with Crouzon Syndrome (CS), an autosomal dominant disease characterized by premature fusion of the skull sutures. This mutation is likely to be pathological since an analogous mutation (N540K) in FGFR3 has been shown to be associated with hypochondroplasia. (Kan S *et al.* (2002) *Am.J.Hum.Genet*;70:472-486) and Lajeunie E *et al.* (2006) *European J Hum Genet*; 14:289-298)

Purity 79.6% by SDS-PAGE and Coomassie blue staining. MW = 38.1kDa.

Specific Activity (Parent lot# 1606092): 6390U/mg, where one unit of FGFR2 (N549H), active activity is defined as 1nmol phosphate incorporated into 500µM (GEEEEYFELVKKKK) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.926mg/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.

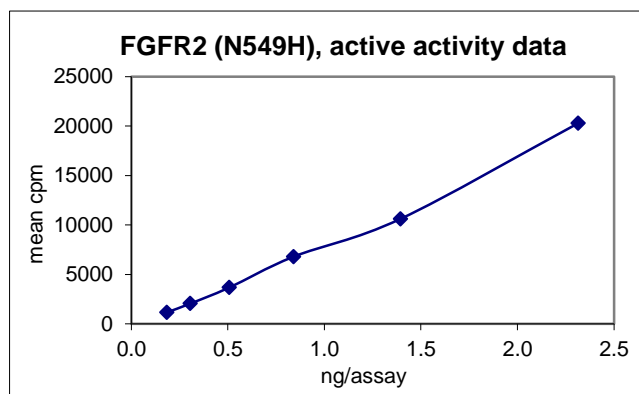
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

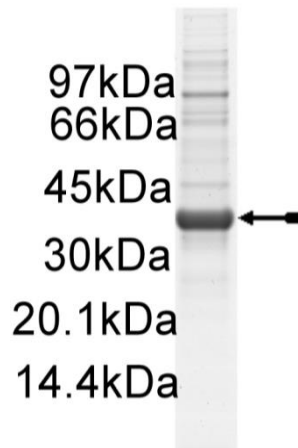
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.2–2.3ng of this lot of enzyme phosphorylated 500µM (GEEEEYFELVKKKK) 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 FGFR2 (N549H) 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 (N549H), 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 concentration of 500 μ M. Add 2.5 μ l of stock per assay point.
3. **FGFR2 (N549H), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–2.3ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of 500 μ M **(GGEEEEYFELVKKKK)**.
3. Add **2.5 μ l (0.2–2.3ng) FGFR2 (N549H), active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P] ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 1 μ l of 30% phosphoric acid.

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FGFR2 (N549H) Sequence Information

<u>Protein</u>	human FGFR2 (N549H)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	D16 of the recombinant protein is equivalent to D456 of native human FGFR2
<u>Accession number</u>	GenBank NM_000141

Recombinant FGFR2 (N549H) amino acid sequence:

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1  MHHHHHHEFK  GLRRQDTPML  AGVSEYELPE  DPKWEFPRDK  LTLGKPLGEG  CFGQVVMAEA
61  VGIDKDKPKE  AVTVAVKMLK  DDATEKDLS  LVSEMEMMKM  IGKHKNIIIN  LGACTQDGPL
121 YVIVEYASKG  NLREYLARR  PPGMEYSYDI  NRVPEEQMTF  KDLVSCTYQL  ARGMEYLASQ
181 KCIHRDLAAR  NVLVTENNV  KIADFGLARD  INNIDYKKT  TNGRLPVKWM  APEALFDRVY
241 THQSDVWSFG  VLMWEIFTLG  GSPYPGIPVE  ELFKLLKEGH  RMDKPANCTN  ELYMMMRDCW
301 HAVPSQRPTF  KQLVEDLDRI  LTLTTNEEYL

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Recombinant FGFR2 (N549H) nucleotide sequence:

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1  atgcatcatc  accatcacca  tgaattcaaa  ggcctacgtc  gacaagacac  ccccatgctg
61  gcaggggtct  ccgagtatga  acttccagag  gacccaaaat  gggagtttcc  aagagataag
121 ctgacactgg  gcaagcccc  gggagaagg  tgctttgggc  aagtggatcat  ggcggaagca
181 gtgggaattg  acaaagacaa  gcccaaggag  gcggtcaccg  tggccgtgaa  gatgttgaaa
241 gatgatgcca  cagagaaaga  ctttctgat  ctggtgtcag  agatggagat  gatgaagatg
301 attgggaaac  acaagaatat  cataaatctt  cttggagcct  gcacacagga  tgggcctctc
361 tatgtcatag  ttgagtatgc  ctctaaaggc  aacctccgag  aatacctccg  agcccgaggg
421 ccaccgggga  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  ggcttcagg  caagtggatg  gctccagaag  cctgtttga  tagagtatac
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901 catgcagtgc  cctccagag  accaacgttc  aagcagttgg  tagaagactt  ggatcgaatt
961 ctcactctca  caaccaatga  ggaatacttg  taa

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Reviewed and approved by site quality representative.

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