eurofins | Discovery Services

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 Ni^{2+}/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 (GGEEEEYFELVKKKK) 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.

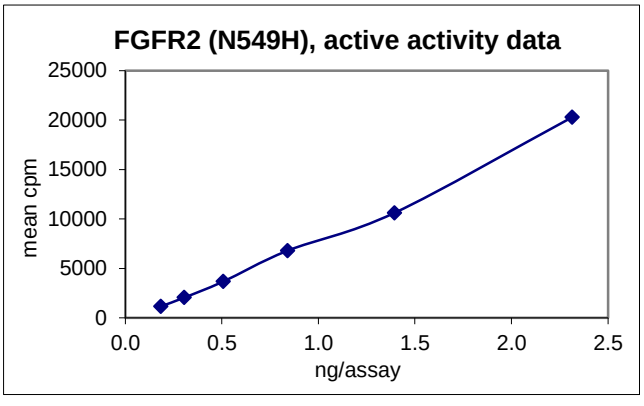
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

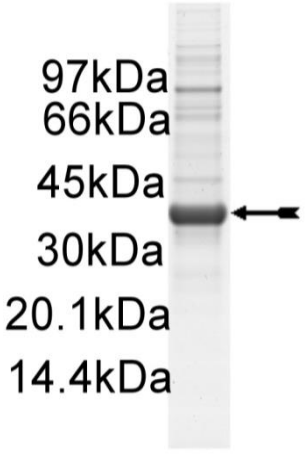
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.2–2.3ng of this lot of enzyme phosphorylated 500µM (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 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.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

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 500µM. Add 2.5µl 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-^{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 500µM **(GGEEEEYFELVKKKK)**.
3. Add **2.5µl (0.2–2.3ng) FGFR2 (N549H), active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P] ATP mixture.
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.

eurofins | Discovery Services

Certificate of Analysis

FGFR2 (N549H) Sequence Information

Protein human FGFR2 (N549H)

Tags *N*-terminal 6His

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

Accession number GenBank NM_000141

Recombinant FGFR2 (N549H) amino acid sequence:

```
  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
```

Recombinant FGFR2 (N549H) nucleotide sequence:

```
  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
```

Reviewed and approved by site quality representative.