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Certificate of Analysis

FGFR4, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-583, 14-583-K, 14-583M
Parent Lot # WAD0126

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 FGFR4 amino acids 442–755, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 75% by SDS-PAGE and Coomassie blue staining. MW = 36.4kDa.

Specific Activity (Parent lot# WAD0126): 1289U/mg, where one unit of FGFR4, 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: 0.46mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

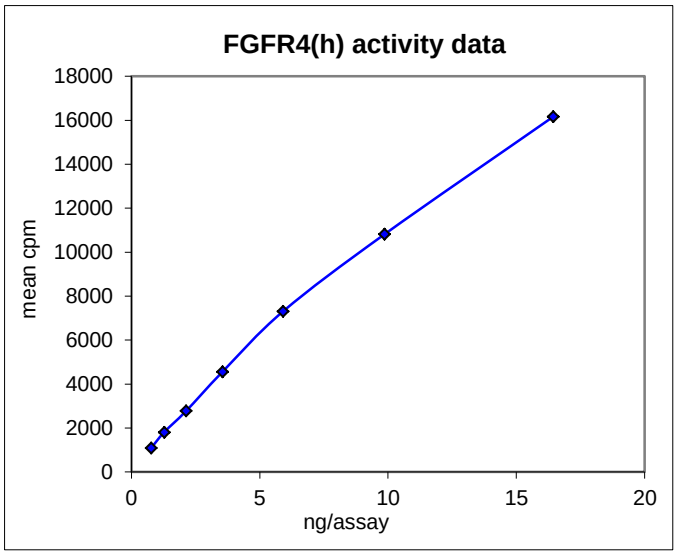
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

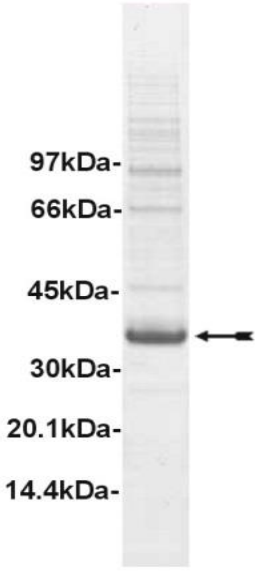
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0.8–16.4ng 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 FGFR4 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 FGFR4, 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

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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 in dH_2O and add 1.25µl of stock per assay point.
3. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Add 2.5µl of stock per assay point.
4. **FGFR4, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.8–16.4ng 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 **poly(Glu, Tyr) (4:1)**.
3. Add 1.25µl of $MnCl_2$.
4. Add 3.75µl of dH_2O.
5. Add **2.5µl (0.8–16.4ng) FGFR4 , 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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FGFR4 Sequence Information

Protein human FGFR4

Tags *N*-terminal 6His

Native sequence G10 of recombinant sequence is equivalent to G442 of native human FGFR4

Accession number GenBank L03840

Recombinant FGFR4 amino acid sequence:

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  1 MHHHHHHEFG PALLAGLVSL DLPLDPLWEF PRDRLVLGKP LGEGCFGQVV RAEAFGMDPA
 61 RPDQASTVAV KMLKDNASDK DLADLVSEME VMKLIGRHKN IINLLGVCTQ EGPLYVIVEC
121 AAKGNLREFL RARRPPGPDL SPDGPRSSEG PLSFPVLVSC AYQVARGMQY LESRKCIHRD
181 LAARNVLVTE DNVMKIADFG LARGVHHIDY YKKTSNGRLP VKWMAPEALF DRVYTHQSDV
241 WSFGILLWEI FTLGGSPYPG IPVEELFSLL REGHRMDRPP HCPPELYGLM RECWHAAPSQ
301 RPTFKQLVEA LDKVLLAVSE EYL
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Recombinant FGFR4 nucleotide sequence:

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  1 atgcatcatc accatcacca tgaattcggc cccgccttgc tcgccggcct cgtgagtcta
 61 gatctacctc tcgacccact atgggagttc ccccgggaca ggctggtgct tgggaagccc
121 ctaggcgagg gctgctttgg ccaggtagta cgtgcagagg cctttggcat ggaccctgcc
181 cggcctgacc aagccagcac tgtggccgtc aagatgctca aagacaacgc ctctgacaag
241 gacctggccg acctggtctc ggagatggag gtgatgaagc tgatcggccg acacaagaac
301 atcatcaacc tgcttggtgt ctgcacccag gaagggcccc tgtacgtgat cgtggagtgc
361 gccgccaagg gaaacctgcg ggagttcctg cgggcccggc gccccccagg ccccgacctc
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481 gcctaccagg tggcccgagg catgcagtat ctggagtccc ggaagtgtat ccaccgggac
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601 ctggcccgcg gcgtccacca cattgactac tataagaaaa ccagcaacgg ccgcctgcct
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841 cactgccccc cagagctgta cgggctgatg cgtgagtgct ggcacgcagc gccctcccag
901 aggcctacct tcaagcagct ggtggaggcg ctggacaagg tcctgctggc cgtctctgag
961 gagtacctct aa
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Reviewed and approved by site quality representative.