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Certificate of Analysis

FGR, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-568, 14-568-K, 14-568M
Parent Lot # 1603539

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 FGR, amino acids 2–end expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 79.7% by SDS-PAGE and Coomassie blue staining. MW = 60.4kDa.

Specific Activity (Parent lot# 1603539): 2800U/mg, where one unit of FGR, 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: 1.109mg/ml of enzyme 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.

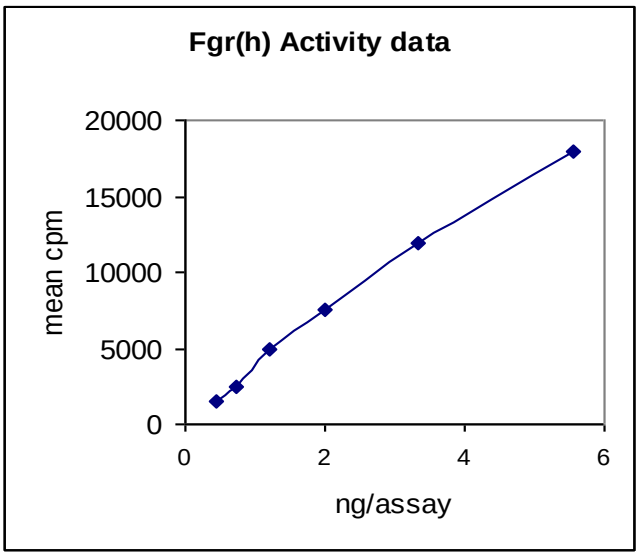
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

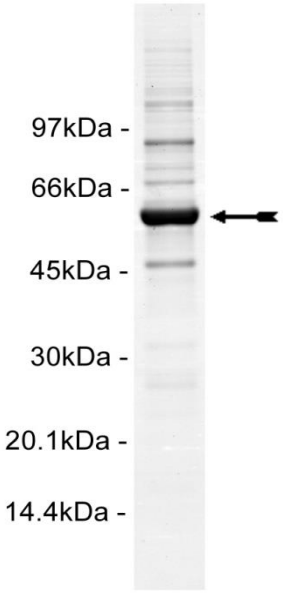
Quality Control Testing

Kinase Assay: 0.4–5.6ng 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 FGR 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 FGR, 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 assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
3. **FGR, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 2-mercaptoethanol, 1mg/ml BSA. Use 0.4–5.6ng 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 to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (0.4–5.6ng) FGR, 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 **Filtermat A**.
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.

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FGR Sequence Information

Protein Human FGR

Tags *N*-Terminal 6His

Native sequence G8 of the recombinant protein is equivalent to G2 of human FGR

Accession number GenBank M19722

Recombinant FGR amino acid sequence:

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  1 MHHHHHHGCV FCKKLEPVAT AKEDAGLEGD FRSYGAADHY GPDPTKARPA SSFAHIPNYS
 61 NFSSQAINPG FLDSGTIRGV SGIGVTLFIA LYDYEARTED DLTFTKGEKF HILNNTEGDW
121 WEARSLSSGK TGCIPSNYVA PVDSIQAEEW YFGKIGRKDA ERQLLSPGNP QGAFLIRESE
181 TTKGAYSLSI RDWDQTRGDH VKHYKIRKLD MGGYYITTRV QFNSVQELVQ HYMEVNDGLC
241 NLLIAPCTIM KPQTLGLAKD AWEISRSSIT LERRLGTGCF GDVWLGTWNG STKVAVKTLK
301 PGTMSPKAFL EEAQVMKLLR HDKLVQLYAV VSEEPIYIVT EFMCHGSLLD FLKNPEGQDL
361 RLPQLVDMAA QVAEGMAYME RMNYIHRDLR AANILVGERL ACKIADFGLA RLIKDDEYNP
421 CQGSKFPIKW TAPEAALFGR FTIKSDVWSF GILLTELITK GRIPYPGMNK REVLEQVEQG
481 YHMPCPPGCP ASLYEAMEQT WRLDPEERPT FEYLQSFLED YFTSAEPQYQ PGDQT
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Recombinant FGR nucleotide sequence:

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   1 atgcatcacc atcaccatca tggctgtgtg ttctgcaaga aattggagcc ggtggccacg
  61 gccaaggagg atgctggcct ggaaggggac ttcagaagct acggggcagc agaccactat
 121 gggcctgacc ccactaaggc ccggcctgca tcctcatttg cccacatccc caactacagc
 181 aacttctcct ctcaggccat caaccctggc ttccttgata gtggcaccat caggggtgtg
 241 tcagggattg gggtgaccct gttcattgcc ctgtatgact atgaggctcg aactgaggat
 301 gacctcacct tcaccaaggg cgagaagttc cacatcctga acaatactga aggtgactgg
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1501 tggcgtctgg acccggagga gaggcctacc ttcgagtacc tgcagtcctt cctggaggac
1561 tacttcacct ccgctgaacc acagtaccag cccggggatc agacatag
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Reviewed and approved by site quality representative.