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Certificate of Analysis

Fes/Fps, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-473, 14-473-K, 14-473M
Parent Lot # 28202U

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 Fes, residues 2–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 83% by SDS-PAGE and Coomassie blue staining. MW = 98kDa.

Specific Activity (Parent lot# 28202U): 83U/mg, where one unit of Fes, 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: 1.18mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.03% Brij-35, 0.1mM EGTA, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

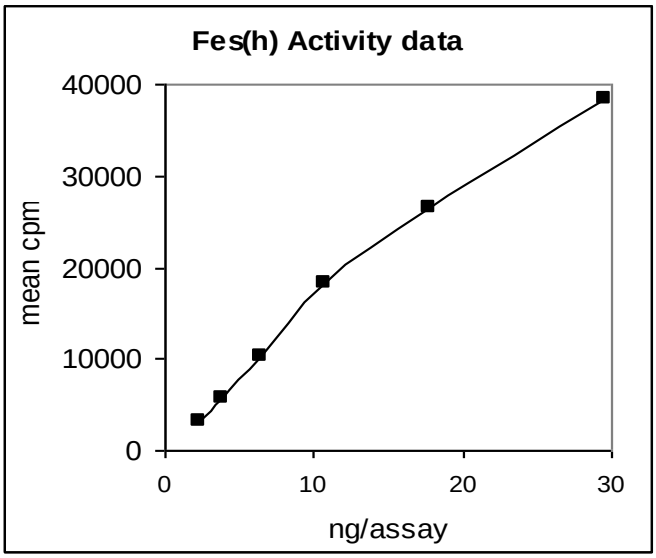
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

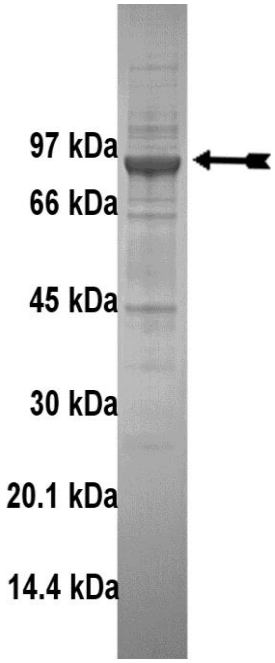
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 2.3–29.5ng 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 identity as Fes 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 Fes, 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 stock per assay point.
3. **Fes, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.3–29.5ng 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 **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (2.3–29.5ng) Fes, active**.
4. Add 5µl dH_20.
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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Certificate of Analysis

Fes Sequence Information

Protein human Fes

Tags *N*-terminal 6His

Native sequence G32 of the recombinant protein is equivalent to G2 of human Fes

Accession number GenBank X06292

Recombinant Fes amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGIRN SGFSSELCSP QGHGVLQQMQ EAELRLLEGM
 61 RKWMAQRVKS DREYAGLLHH MSLQDSGGQS RAISPDSPIS QSWAEITSQT EGLSRLLRQH
121 AEDLNSGPLS KLSLLIRERQ QLRKTYSEQW QQLQQELTKT HSQDIEKLKS QYRALARDSA
181 QAKRKYQEAS KDKDRDKAKD KYVRSLWKLF AHHNRYVLGV RAAQLHHQHH HQLLLPGLLR
241 SLQDLHEEMA CILKEILQEY LEISSLVQDE VVAIHREMAA AAARIQPEAE YQGFLRQYGS
301 APDVPPCVTF DESLLEEGEP LEPGELQLNE LTVESVQHTL TSVTDELAVA TEMVFRRQEM
361 VTQLQQELRN EEENTHPRER VQLLGKRQVL QEALQGLQVA LCSQAKLQAQ QELLQTKLEH
421 LGPGEPPPVL LLQDDRHSTS SSEQEREGGR TPTLEILKSH ISGIFRPKFS LPPPLQLIPE
481 VQKPLHEQLW YHGAIPRAEV AELLVHSGDF LVRESQGKQE YVLSVLWDGL PRHFIIQSLD
541 NLYRLEGEGF PSIPLLIDHL LSTQQPLTKK SGVVLHRAVP KDKWVLNHED LVLGEQIGRG
601 NFGEVFSGRL RADNTLVAVK SCRETLPPDL KAKFLQEARI LKQYSHPNIV RLIGVCTQKQ
661 PIYIVMELVQ GGDFLTFLRT EGARLRVKTL LQMVGDAAAG MEYLESKCCI HRDLAARNCL
721 VTEKNVLKIS DFGMSREEAD GVYAASGGLR QVPVKWTAPE ALNYGRYSSE SDVWSFGILL
781 WETFSLGASP YPNLSNQQTR EFVEKGGRLP CPELCPDAVF RLMEQCWAYE PGQRPSFSTI
841 YQELQSIRKR HRKLVEKY
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Recombinant Fes nucleotide sequence:

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   1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
  61 tattttcagg gcgccatggg gatccggaat tcgggcttct cttccgagct gtgcagcccc
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2521 taccaggagc tgcagagcat ccgaaagcgg catcggaagc ttgtcgagaa gtactag
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