

Certificate of Analysis

Fer, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-605, 14-605-K, 14-605M

Parent Lot # 1798041

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 Fer amino acids 541–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 84.4% by SDS-PAGE and Coomassie blue staining. MW = 35.6kDa.

Specific Activity (Parent lot# 1798041): 863U/mg, where one unit of Fer, active activity is defined as 1nmol phosphate incorporated into 250μM (KKKSPGEYVNIEFG) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.319mg/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. 20mM sodium β-glycerophosphate, 10mM sodium fluoride, 0.25mM sodium vanadate. Frozen solution.

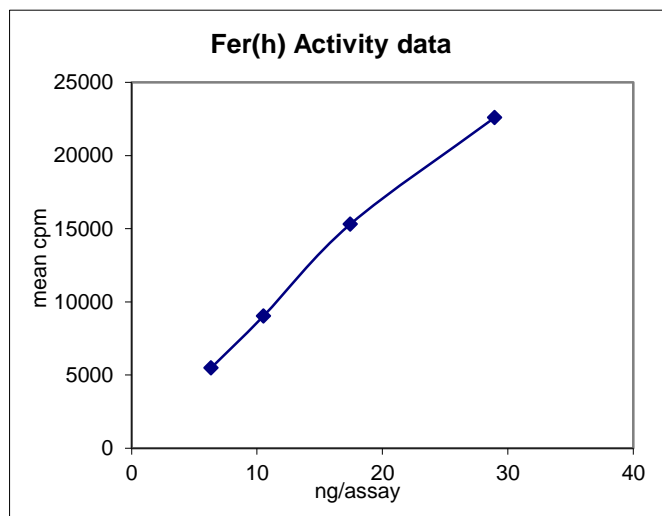
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

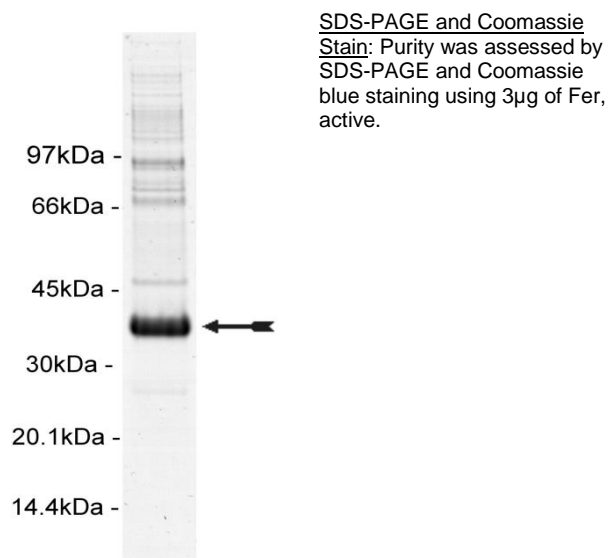
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 6–29ng of this lot of enzyme phosphorylated 250μM (KKKSPGEYVNIEFG) 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 Fer with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKSPGEYVNIEFG):** Use at a final assay concentration of 250 μ M. Prepare a 2.5mM stock and add 2.5 μ l of stock per assay point.
3. **MnCl₂:** Use at a final assay concentration of 1mM. Prepare a 10mM stock and add 2.5 μ l of stock per assay point.
4. **Fer, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–29ng per assay point.
5. **[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 **(KKKSPGEYVNIEFG)**.
3. Add **2.5 μ l (6–29ng) Fer, active**.
4. Add 2.5 μ l of 10mM MnCl₂.
5. Add 2.5 μ l dH₂O.
6. Add 10 μ l of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
9. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
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 1 μ l of 30% phosphoric acid.

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Fer Sequence Information

<u>Protein</u>	human Fer
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	K29 of the recombinant protein is equivalent to K541 of human Fer
<u>Accession number</u>	GenBank NM_005246

Recombinant Fer amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMGSKS GVVLLNPIPK DKKWILSHED VILGELLGKG
61 NFGEVYKGTI KDKTSVAVKT CKEDLPQELK IKFLQEAKIL KQYDHPNIVK LIGVCTQRQP
121 VYIIMELVSG GDFLTFLRRK KDELKCLKLV KFSLDAAAGM LYLESKNCIH RDLAARNCLV
181 GENNVLKISD FGMSRQEDGG VYSSSGLKQI PIKWTAPEAL NYGRYSSES D VWSFGILLWE
241 TFSLGVCYPY GMTNQQAREQ VERGYRMSAP QHCPEDISKI MMKCWDYKPE NRPKFSELQK
301 ELTIKKRKLK

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Recombinant Fer nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tatttttcagg gcgccatggg atccaaatca ggtgtagttc tgctgaatcc tattcctaag
121 gacaagaaat ggattctcag tcatgaagat gtcataattgg gagaattact gggcaaggga
181 aatttttggtg aagtatataa gggcacatta aaggacaaaa cttctgttgc tgttaaaaca
241 tgtaaagaag atcttcctca ggaattgaaa ataaaatttt tacaagaagc caaaattctc
301 aagcaatatg atcatcccaa tattgtcaaa cttataggag tttgcacaca aagacagcct
361 gtctacatca ttatggaact ggtttcagga ggtgatttcc tcacctttct gagaaggaag
421 aaggatgaac taaaactcaa acagtttagtg aaattttcat tagacgtgc tgctggtatg
481 ttgtatctcg agagtaaaaa ctgtatacac agggaccttg ctgcaagaaa ctgcctggta
541 ggtgaaaata atgttctgaa aatcagtgac tttggaatgt ctcgtcaaga ggatggtgga
601 gtgtattcat cttctggctt aaagcagatt cccattaaat ggacagcacc ggaagctctt
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901 gagctcacta tcatcaagag aaaactcaca tag

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