

# Fes Kinase

✓ 5 µg

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New 02/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant full length human Fes kinase, supplied as a GST fusion protein.

**Background:** Fes/Fps and Fer are the only two members of a unique family of cytoplasmic protein tyrosine kinases (1,2). Fps and Fer contain a central Src homology-2 (SH2) domain and a carboxy-terminal tyrosine kinase catalytic domain. They are structurally distinguished from other members of cytoplasmic protein tyrosine kinase subfamilies by the presence of amino-terminal Fer/CIP4 homology and coiled-coil domains (3). Fes/Fps was originally identified as an oncogene from avian (Fps) and feline (Fes) retroviruses. Human c-Fes has been implicated in myeloid, vascular endothelial and neuronal cell differentiation. Mutations may activate the Fps kinase and thereby contribute to cancer (4). However, recent data strongly suggests that the c-Fes protein-tyrosine kinase is a tumor suppressor rather than a dominant oncogene in colorectal cancer (5).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system using sf9 cells and a recombinant virus encoding full length human Fes (Met1-Arg822) (GenBank Accession No. NM\_002005) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

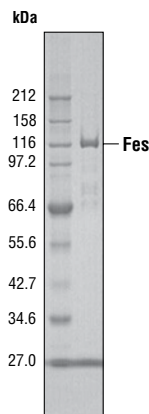


Figure 1. The purity of the GST-Fes fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

**Quality Control:** The theoretical molecular weight of the GST-Fes fusion protein is 125 kDa. The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. Fes kinase activity was determined using a radiometric assay [Fig.2].

**Background References:**

- (1) Smithgall, T.E. et al. (1998) *Crit. Rev. Oncog.* 9, 43–62.
- (2) Greer, P. (2002) *Nat. Rev. Mol. Cell Biol.* 3, 278–289.
- (3) Sangrar, W. et al. (2005) *Cancer Res.* 65, 3518–3522.
- (4) Ley, T.J. et al. (2003) *Proc. Natl. Acad. Sci. USA* 100, 14275–14280.
- (5) Delfino, F.J. et al. (2006) *J. Biol. Chem.* 281, 8829–8835.

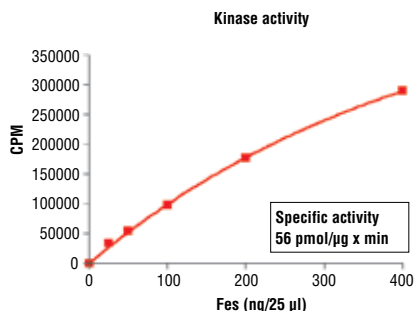


Figure 2. Fes kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP. Substrate: Poly (Glu:Tyr, 4:1) 400 ng/µL, and recombinant Fes: variable.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

**Companion Products:**

- Kinase Buffer (10X) #9802
- ATP (10 mM) #9804
- Serine/Threonine Kinase Substrate Screening Kit #7400

## Protocol for Fes Kinase Assay

**Note:** Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

### A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (10X)**  
50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM  $MgCl_2$   
0.5 mM DTT
- 2.** ATP (10 mM) #9804
- 3.**  $^{32}P$ - $\gamma$ ATP
- 4.** Poly (Glu:Tyr, 4:1) (1  $\mu$ g/ $\mu$ l)

### B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
- 2.** Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute Fes protein to 40 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10  $\mu$ l diluted Fes kinase solution, 10  $\mu$ l Poly (Glu:Tyr, 4:1) (1  $\mu$ g/ $\mu$ l), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

- 5 mM MOPS, pH 7.2
  - 2.5 mM  $\beta$ -glycerophosphate
  - 1 mM EGTA
  - 4 mM  $MgCl_2$
  - 0.05 mM DTT
  - 400 ng/ $\mu$ l Poly (Glu:Tyr, 4:1)
- 6.** After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
  - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
  - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
  - 9.** Count samples in a scintillation counter.

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