

FGF Receptor 4 Kinase

✓ 5 µg

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

rev. 12/06/05

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

Description: Purified recombinant human FGFR-4 kinase (Arg391-Thr802), supplied as a GST fusion protein.

Background: Fibroblast growth factors (FGFs) produce mitogenic and angiogenic effects in target cells by signaling through cell surface receptor tyrosine kinases. There are four members of the FGF receptor family: FGFR-1 (flg), FGFR-2 (bek, KGFR), FGFR-3 and FGFR-4. Each receptor contains an extracellular ligand binding domain, a trans-membrane domain and a cytoplasmic kinase domain (1). Following ligand binding and dimerization, the receptors are phosphorylated at specific tyrosine residues (2). Seven tyrosine residues in the cytoplasmic tail of FGFR-1 can be phosphorylated: Tyr463, Tyr583, Tyr585, Tyr653, Tyr654, Tyr730 and Tyr766. Tyrosines 653 and 654 are important for catalytic activity of activated FGFR and are essential for signaling (3). The other phosphorylated tyrosine residues may provide docking sites for downstream signaling components such as Crk and PLCγ (4,5).

Source/Purification: The GST-kinase fusion protein was produced using a baculovirus expression system with a construct expressing human FGFR-4 (Arg391-Thr802) (GenBank accession No. NM_002011) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-FGFR-4 fusion protein is 75 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. FGFR-4 kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure FGFR-4 activity using HTScan™ FGF Receptor 4 Kinase Assay Kit #7737 [Fig.3].

Background References:

- (1) Powers, C.J. et al. (2000) *Endocr. Relat. Cancer* 7, 165–197.
- (2) Reilly, J.F. and Maher, P.A. (2001) *J. Cell Biol.* 275, 7771–7778.
- (3) Mohammadi, M. et al. (1996) *Mol. Cell. Biol.* 16, 977–989.
- (4) Mohammadi, M. et al. (1991) *Mol. Cell. Biol.* 11, 5068–5078.
- (5) Larsson, H. et al. (1999) *J. Biol. Chem.* 274, 25726–25734.

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 8.0; 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20% glycerol. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ FGF Receptor 4 Kinase Assay Kit #7737

PYK2 (Tyr402) Biotinylated Peptide #1315

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

HTScan™ Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Staurosporine #9953

Tyrosine Kinase Substrate Screening Kit #7450

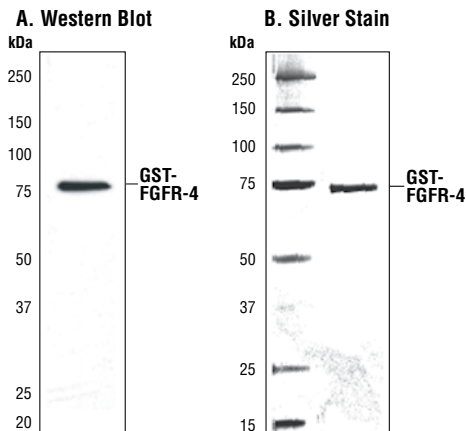


Figure 1. The purity of the GST-FGFR-4 fusion protein was analyzed using SDS/PAGE followed by anti-FGFR-4 Western blot (A) or Silver stain (B).

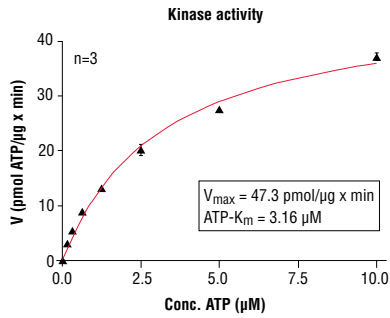


Figure 2. FGFR-4 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: PolyEY, 2 µg/50 µl and 100 ng/50 µl Recombinant FGFR-4.

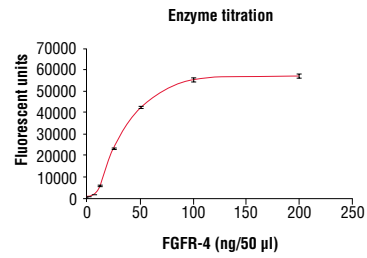


Figure 3. Dose dependence curve of FGFR-4 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1315) by FGFR-4 kinase. In a 50 µl reaction, increasing amounts of FGFR-4 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIATM is a registered trademark of PerkinElmer, Inc.)

Protocol for FGF Receptor 4 Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

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. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411
5. HTScan™ Tyrosine Kinase Buffer (4X) #9805
6. ATP (10 mM) #9804
7. PYK2 (Tyr402) Biotinylated Peptide #1315
8. DTT (1000X, 1.25 M)
9. DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
10. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
11. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH₂O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µM).
2. Immediately transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 10 µl of DTT (1.25 M) to 2.5 ml of 4X HTScan™ Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM MgCl₂, 20 mM MnCl₂, 12 µM Na₃VO₄) to make DTT/Kinase buffer.
5. Transfer 1.25 ml of DTT/Kinase buffer to enzyme tube to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

- 60 mM HEPES pH 7.5
 - 5 mM MgCl₂
 - 5 mM MnCl₂
 - 3 µM Na₃VO₄
 - 1.25 mM DTT
 - 200 µM ATP
 - 1.5 µM peptide
 - 50 ng FGF Receptor 4 Kinase
8. Incubate reaction plate at room temperature for 30 minutes.
 9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
 10. Transfer 25 µl of each reaction and 75 µl of dH₂O/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
 11. *Wash three times with 200 µl/well PBS/T.
 12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.

Please note: This protocol was validated using a PYK2 (Tyr402) Biotinylated Peptide and Phospho-Tyrosine Mouse mAb (P-Tyr-100) diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
 13. Incubate at room temperature for 120 minutes.
 14. *Wash three times with 200 µl/well PBS/T.
 15. Dilute Europium labeled anti-mouse IgG 1:500 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
 16. Incubate at room temperature for 30 minutes.
 17. *Wash five times with 200 µl/well PBS/T.
 18. Add 100 µl/well DELFIA® Enhancement Solution.
 19. Incubate at room temperature for 5 minutes.
 20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com