

VEGF Receptor 3 Kinase

✓ 5 µg

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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 VEGFR-3 kinase (Asn799-Arg1298), supplied as a GST fusion protein.

Background: Vascular endothelial growth factor receptor 3 (VEGFR-3) is a 170 kDa membrane receptor tyrosine Kinase. All of the VEGF receptors are characterized by seven extracellular immunoglobulin (Ig)-like domains followed by a membrane-spanning domain and a conserved intracellular tyrosine kinase domain (1). VEGFR-3 is largely restricted to the lymphatic endothelium in adult tissue and is thought to control lymphangiogenesis (1,2). Binding of VEGF-C/VEGF-D to VEGFR-3 results in transphosphorylation of tyrosine residues in its intracellular domain, recruitment of signaling molecules, and activation of ERK1/2 and Akt signaling cascades (1,3).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human VEGFR-3 (Asn799-Arg1298) (GenBank accession No. NM_002020) 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-VEGFR-3 fusion protein is 86 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. VEGFR-3 kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure VEGFR-3 activity using HTScan™ VEGFR-3 Kinase Assay Kit #7791 [Fig.3].

Background References:

- (1) Robinson, C.J. and Stringer, S.E. (2001) *J. Cell Sci.* 114, 853–865.
- (2) Valtola, R. et al. (1999) *Am. J. Pathol.* 154, 1381–1390.
- (3) Saharinen, P. and Petrova, T.V. (2004) *Ann. N.Y. Acad. Sci.* 1014, 76–87.

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:

Tyrosine Kinase Substrate Screening Kit #7450

MET (Tyr1253) Biotinylated Peptide #1367

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

Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Staurosporine #9953

HTScan™ VEGFR-3 Kinase Assay Kit #7791

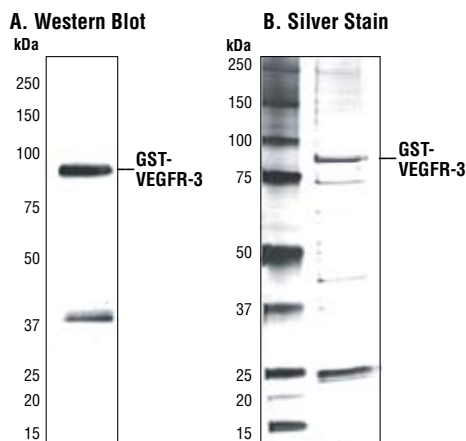


Figure 1. The purity of the GST-VEGFR-3 fusion protein was analyzed using SDS/PAGE followed by anti-VEGFR-3 Western blot (A) or Silver stain (B).

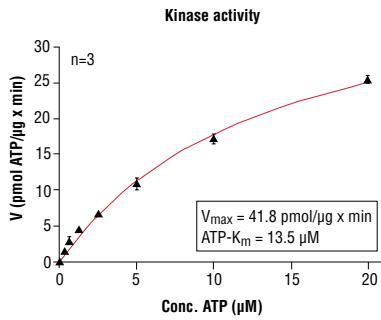


Figure 2. VEGFR-3 kinase activity was measured in a radioisotopic filter binding assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: PolyEY, 5 µg/50 µl and 100 ng/50 µl Recombinant VEGFR-3.

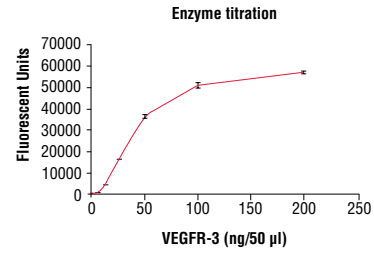


Figure 3. Dose dependence curve of VEGFR-3 kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1367) by VEGFR-3 kinase. In a 50 µl reaction, increasing amounts of VEGFR-3 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIATM is a registered trademark of PerkinElmer, Inc.)

Protocol for VEGF Receptor 3 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. MET (Tyr1253) Biotinylated Peptide #1367
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]=8.0 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
 100 ng VEGF Receptor 3 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 MET (Tyr1253) 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