

#7407 Store at -80°C

VEGF Receptor 2 Kinase

✓ 5 µg

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

Description: Purified recombinant human VEGFR-2 (Val789-Val1356) kinase, supplied as a GST fusion protein.
Background: Vascular endothelial growth factor receptor 2 (VEGFR-2, KDR, Flk-1) is a major receptor transducing VEGF-induced signaling in endothelial cells. Upon ligand binding, VEGFR-2 undergoes autophosphorylation and becomes activated (1). Major autophosphorylation sites of VEGFR-2 are located in the kinase insert domain (Tyr951/996) and in the tyrosine kinase catalytic domain (Tyr1054/1059) (2). Activation of the receptor leads to rapid recruitment of adaptor proteins, including Shc, GRB2, PI-3 kinase, Nck and the protein tyrosine phosphatases SHP-1 and SHP-2 (3). The phosphorylation of Tyr1212 provides a docking site for Grb2 binding and phospho-Tyr1175 binds with the p85 subunit of PI-3 kinase and PLCγ, as well as Shb (5,6). Signaling from VEGFR-2 is necessary for the execution of VEGF-stimulated proliferation, chemotaxis and sprouting, as well as survival of cultured endothelial cells *in vitro* and angiogenesis *in vivo* (4).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human VEGFR-2 (Val789-Val1356) (GenBank Accession No. NM_002253) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

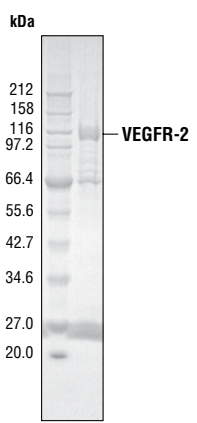


Figure 1. The purity of the GST-VEGFR-2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-VEGFR-2 fusion protein is 110 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. VEGFR-2 kinase activity was determined using a radiometric assay [Fig.2].

- Background References:**
- (1) Meyer, M. et al. (1999) *EMBO J.* 18, 363–374.
 - (2) Dougher-Vermazen, M. et al. (1994) *Biochem. Biophys. Res. Commun.* 205, 728–738.
 - (3) Kroll, J. and Waltenberger, J. (1997) *J. Biol. Chem.* 272, 32521–32527.
 - (4) Karkkainen, M.J. and Petrova, T. (2000) *Oncogene* 19, 5598–5605.
 - (5) Rahimi, N. et al. (2000) *J. Biol. Chem.* 275, 16986–16992.
 - (6) Claesson-Welsh, L. (2003) *Biochem. Soc. Transact.* 31, 20–24.

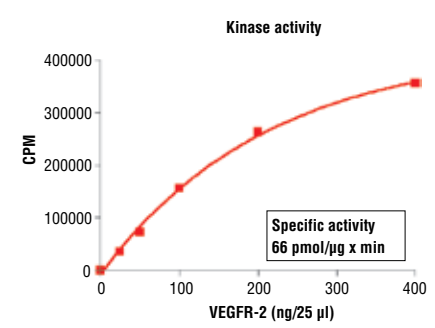


Figure 2. VEGFR-2 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT, 50 µM ATP. Substrate: MBP 200 ng/µL, and recombinant VEGFR-2: variable.

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:
HTScan® Tyrosine Kinase Buffer (4X) #9805
ATP (10 mM) #9804
Tyrosine Kinase Substrate Screening Kit #7450

Protocol for VEGF Receptor 2 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)**
40 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM EDTA
40 mM $MgCl_2$
0.5 mM DTT
- 2.** ATP (10 mM) #9804
- 3.** ^{32}P - γ ATP
- 4.** MBP (0.5 μ g/ μ l)

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from $-80^\circ C$ to ice. Allow enzyme to thaw on ice.
- 4.** Dilute VEGFR-2 kinase protein (100 ng/ μ l concentration) to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted VEGFR-2 kinase solution, 10 μ l MBP (0.5 μ g/ μ l), and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 4 mM MOPS, pH 7.2
 - 2.5 mM β -glycerophosphate
 - 1 mM EGTA
 - 0.4 mM EDTA
 - 4 mM $MgCl_2$
 - 0.05 mM DTT
 - 200 ng/ μ L MBP
- 6.** After 15 minutes terminate reaction by spotting 20 μ 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.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: drugdiscovery@cellsignal.com.