

#2472 Store at -20°C

VEGF Receptor 2 Antibody



✓ 100 µl
(10 Western mini-blot)

Orders ■ 877-616-CELL (2355)
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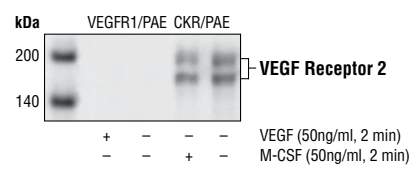
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Transfected	H, M, (R)	210, 230 kDa	Rabbit

Background: Vascular endothelial growth factor receptor 2 (VEGFR2, KDR, Flk-1) is a major receptor transducing VEGF-induced signaling in endothelial cells. Upon ligand binding, VEGFR2 undergoes autophosphorylation and becomes activated (1). Major autophosphorylation sites of VEGFR2 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 (1,4,5). Signaling from VEGFR2 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* (6-8).

Specificity/Sensitivity: VEGF Receptor 2 Antibody detects transfected levels of VEGF-2 receptors. It does not cross-react with other members of VEGF receptor family.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues near the carboxy-terminal sequence of human VEGF receptor 2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from PAE cells overexpressing VEGF receptor 1 and chimeric VEGF receptor 2 respectively, using VEGF Receptor 2 Antibody. CKR/PAE cells overexpress chimeric receptors containing M-CSF receptor extracellular domain and VEGF Receptor 2 transmembrane and cytoplasmic domains (5).

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) Takahashi, T. et al. (2001) *EMBO J.* 20, 2768-2778.
- (5) Holmqvist, K. et al. (2004) *J. Biol. Chem.* 279, 22267-22275.
- (6) Karkkainen, M.J. and Petrova, T. (2000) *Oncogene* 19, 5598-5605.
- (7) Rahimi, N. et al. (2000) *J. Biol. Chem.* 275, 16986-16992.
- (8) Claesson-Welsh, L. (2003) *Biochem. Soc. Trans.* 31, 20-24.

Entrez-Gene ID # 3791
Swiss-Prot Acc. # P35968

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
Western blotting 1:1000

- Companion Products:**
- Phospho-VEGF Receptor 2 (Tyr951) Antibody #2471
 - Phospho-VEGF Receptor 2 (Tyr996) Antibody #2474
 - Phospho-VEGF Receptor 2 (Tyr1212) (11A3) Rabbit mAb #2477
 - Phospho-VEGF Receptor 2 (Tyr951) (7H11) Mouse mAb #2476
 - Phospho-VEGF Receptor 2 (Tyr1175) (19A10) Rabbit mAb #2478
 - VEGF Receptor 2 (55B11) Rabbit mAb #2479
 - VEGF-C Antibody #2445
 - VEGF-B Antibody #2463
 - VEGF Receptor 3 Antibody #2485
 - Phospho-VEGF Receptor 2 (Tyr951) (15D2) Rabbit mAb #4991
 - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.