#8696 Store at -20°C

Angiogenesis Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb	4060	20 µl	60 kDa	Rabbit IgG
Phospho-FAK (Tyr397) (D20B1) Rabbit mAb	8556	20 µl	125 kDa	Rabbit IgG
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP® Rabbit mAb	4511	20 µl	43 kDa	Rabbit IgG
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb	4370	20 µl	44, 42 kDa	Rabbit IgG
Phospho-PLCγ1 (Ser1248) (D25A9) Rabbit mAb	8713	20 µl	150 kDa	Rabbit IgG
Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb	6943	20 µl	60 kDa	Rabbit IgG
Phospho-VEGF Receptor 2 (Tyr1175) (19A10) Rabbit mAb	2478	20 µl	230 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Angiogenesis Antibody Sampler Kit provides an economical means to investigate the angiogenic pathway downstream of VEGFR2. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Vascular endothelial growth factor receptor 2 (VEGFR2, KDR, Flk-1) is a major receptor for VEGF-induced signaling in endothelial cells. Upon ligand binding, VEGFR2 undergoes autophosphorylation and becomes activated (1). Signaling from VEGFR2 is necessary for angiogenesis *in vivo* (2-4). Activation of the receptor leads to rapid recruitment of adaptor proteins, including Shc, GRB2, PI3 kinase, NCK, and the protein tyrosine phosphatases SHP-1 and SHP-2 (5). Phosphorylation of VEGFR2 at Tyr1212 provides a docking site for GRB2 binding and phosphorylation at Tyr1175 binds the p85 subunit of PI3 kinase and PLCγ (1,5,6). Activation of VEGFR2 during angiogenesis leads to signaling through multiple downstream kinase pathways including Akt, Src, FAK, p38, and Erk1/2 (2,7).

Specificity/Sensitivity: Each antibody in the Angiogenesis Antibody Sampler Kit recognizes the phosphorylated form of its specific target. Phospho-FAK (Tyr397) (D20B1) Rabbit mAb may cross-react with overexpressed tyrosine phosphorylated proteins such as EGFR. Phospho-Src Family (Tyr416) (D49G4) Rabbit mAb may cross-react with other Src family members or overexpressed phosphorylated RTKs. Phospho-VEGFR2 (Tyr1175) (19A10) Rabbit mAb may cross-react with VEGFR1.

Source/Purification: Rabbit monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser473 of human Akt protein, Tyr397 of human FAK protein, Thr180/Tyr182 of human p38 MAPK protein, Thr202/Tyr204 of human p44 MAPK protein, Ser1248 of human PLCγ1 protein, Tyr416 of human Src protein, and Tyr1175 of human VEGFR2 protein.

Background References:

(1) Meyer, M. et al. (1999) *EMBO J* 18, 363-74.
(2) Karkkainen, M.J. and Petrova, T.V. (2000) *Oncogene* 19, 5598-605.
(3) Rahimi, N. et al. (2000) *J Biol Chem* 275, 16986-92.
(4) Claesson-Welsh, L. (2003) *Biochem Soc Trans* 31, 20-4.
(5) Holmqvist, K. et al. (2004) *J Biol Chem* 279, 22267-75.
(6) Takahashi, T. et al. (2001) *EMBO J* 20, 2768-78.
(7) Le Boeuf, F. et al. (2004) *J Biol Chem* 279, 39175-85.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.