

#4221 Store at -20°C

Phospho-Tie2 (Tyr992) Antibody



100 µl
 (10 Western mini-blot)

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

rev. 02/01/08

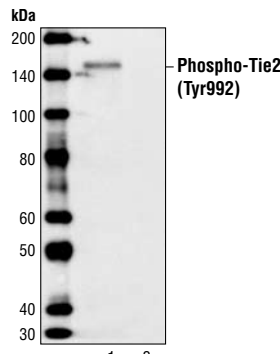
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Transfected	H, (M)	160 kDa	Rabbit

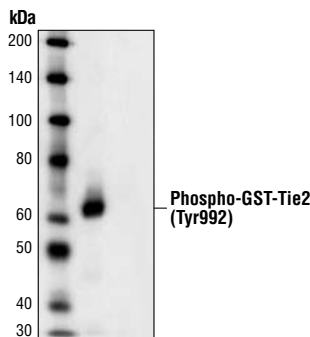
Background: Tie2/Tek is a receptor tyrosine kinase (RTK) expressed almost exclusively on endothelial cells. It is critical for vasculogenesis and could be important for maintaining endothelial cell survival and integrity in adult blood vessels as well as tumor angiogenesis (1-3). A family of ligands known as the angiopoietins binds to Tie2. Interestingly, these ligands appear to have opposing actions, as Angiopoietin-1 (Ang1) and Angiopoietin-4 (Ang4) stimulate tyrosine phosphorylation of Tie2, whereas Angiopoietin-2 (Ang2) and Angiopoietin-3 (Ang3) can inhibit this phosphorylation (4,5). Downstream signaling components, including Grb2, Grb7, Grb14, Shp2, the p85 subunit of phosphatidylinositol 3-kinase and p56/Dok-2, interact with Tie2 in a phosphotyrosine-dependent manner through their SH2 or PTB domains (6,7). Tyr992 is located on the putative activation loop of Tie2 and is a major autophosphorylation site (8). Phosphorylation of Ser1119 at the carboxy terminus of Tie2 results in an increase of Tie2 kinase activity (personal communication with Dr. Chris Kontos, Duke University).

Specificity/Sensitivity: Phospho-Tie2 (Tyr992) Antibody detects transfected levels of Tie2 protein only when phosphorylated at Tyr992.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Tyr992 of human Tie2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from 293 cells, transfected with wild-type Tie2 (lane 1) or mock-transfected (lane 2) using Phospho-Tie2 (Tyr992) Antibody. Tie2 is constitutively active when transfected into 293 cells.



Western blot analysis of extracts from Sf9 cells overexpressing GST-human Tie2 kinase domain fusion proteins, wild-type (lane 1) or kinase-dead (lane 2) using Phospho-Tie2 (Tyr992) Antibody (upper) or Tie2 antibody (lower). The wild-type Tie2 kinase domain is constitutively phosphorylated when overexpressed in Sf9 cells. The molecular weight of GST-Tie2 fusion is approximately 65 kDa.

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:

- Tie2 (AB33) Mouse mAb #4224
- Phospho-Tie2 (Ser1119) Antibody #4226
- 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

Background References:

- (1) Ward, N.L. and Dumont, D.J. (2002) *Semin. Cell Dev. Biol.* 13, 19–27.
- (2) Jones, N. and Dumont, D.J. (2000) *Cancer Metastasis Rev.* 19, 13–17.
- (3) Partanen, J. and Dumont, D.J. (1999) *Curr. Top. Microbiol. Immunol.* 237, 159–172.
- (4) Ellis, L.M. et al. (2002) *Oncology* 16, 31–35.
- (5) Koh, G.Y. et al. (2002) *Exp. Mol. Med.* 34, 1–11.
- (6) Jones, N. et al. (1999) *J. Biol. Chem.* 274, 30896–30905.
- (7) Jones, N. et al. (2003) *Mol. Cell. Biol.* 23, 2658–2668.
- (8) Murray, B. W. et al. (2001) *Biochem.* 40, 10243–10253.

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—DELFI[®]
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.