Rabbit (polyclonal) Anti-Tie2 [pY^{1108}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-1313G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated Tie2. The final product is generated by affinity chromatography using a Tie2-derived peptide that is phosphorylated at tyrosine 1108.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human Tie2 that contains tyrosine 1108.
Target Summary:	Tie2 (also known as TEK) is a 140 kDa endothelial receptor tyrosine kinase that regulates signaling events mediating embryonic vascular development and tumor angiogenesis. Disruption of Tie2 receptor signaling results in embryonic lethality due to vascular hemorrhage and impaired cardiac development. Tie2 receptor consists of an extracellular region that contains three EGF-like modules flanked by two immunoglobulin (Ig)-like domains, three fibronectin type III (FNIII) repeats, and a cytoplasmic region that contains tyrosine kinase domains including several phosphorylation and binding sites. Phosphorylation of Tie2 at distinct tyrosine residues plays a critical role in vascular development and hematopoiesis including tyrosine 992 in the activation loop which primes the phosphorylation of Tie2 on other tyrosine residues including tyrosine 1102 (also known as tyrosine 1100) and 1108 (also referred to as tyrosine 1106). Tyrosine 1108 is an angiopoietin (Ang 1)-dependent autophosphorylation site that mediates binding and phosphorylation of Dok-R and plays an important role in linking Ang-1 stimulation with downstream cell migration signaling in endothelial cells.
Reactivity:	Human Tie2. Mouse and rat Tie2 (100%) have not been tested but are expected to react.
Applications:	The antibody has been used for Western blotting applications.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Controls Used:	Human umbilical vein endothelial (HUVAC) cells plated on fibronectin.

This product is for research use only. Not for use in diagnostic procedures.

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Related Products:

Antibodies:

Tie2 [pY^{992}], Cat. # 44-1310G
VEGFR2 [pY^{949}] , Cat # 44- 1041G
VEGFR2 [pY^{951}], Cat. # 44-1040G
VEGFR2 [pY^{1054}], Cat # 44-1046G
VEGFR2 [pY$^{1054/1059}$], Cat. # 44-1047G
FAK [pY^{397}], Cat. # 44-624G
FAK [pY^{576}], Cat. # 44-652G
FAK [pS^{843}], Cat. # 44-594
Integrin α4 [pS^{988}], Cat. # 44-864
Integrin β1 [pS^{785}], Cat. # 44-870
Integrin β1 [pTpT$^{788/789}$], Cat. # 44-872
Integrin β3 [pY^{773}], Cat. # 44-876
Integrin β3 [pY^{785}], Cat. # 44-878
VE-cadherin [pY^{731}], Cat. # 44-1145G

References:

Harfouche, R. and S.N. Hussian (2006) Signaling and regulation of endothelial cell survival by angiopoietin-2. Am. J. Physiol. Heart Circ. Physiol. 291(4):H1635-H1645.

Tsigkos, S., et al. (2006) Regulation of Ang2 release by PTEN/PI3-kinase/Akt in lung microvascular endothelial cells. J. Cell Physiol. 207(2):506-511.

Cascone, I., et al. (2005) Stable interaction between alpha5beta1 integrin and Tie2 tyrosine kinase receptor regulates endothelial cell response to Ang-1. J. Cell Biol. 170(6):993-1004.

Valable, S., et al. (2003) Angiopoietin-1-induced PI3-kinase activation prevents neuronal apoptosis. FASEB J. 17(3):443-445.

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Murray, B.W., et al. (2001) Mechanistic effects of autophosphorylation on receptor tyrosine kinase catalysis: enzymatic characterization of Tie2 and phospho-Tie2. Biochemistry 40(34):10243-10253.

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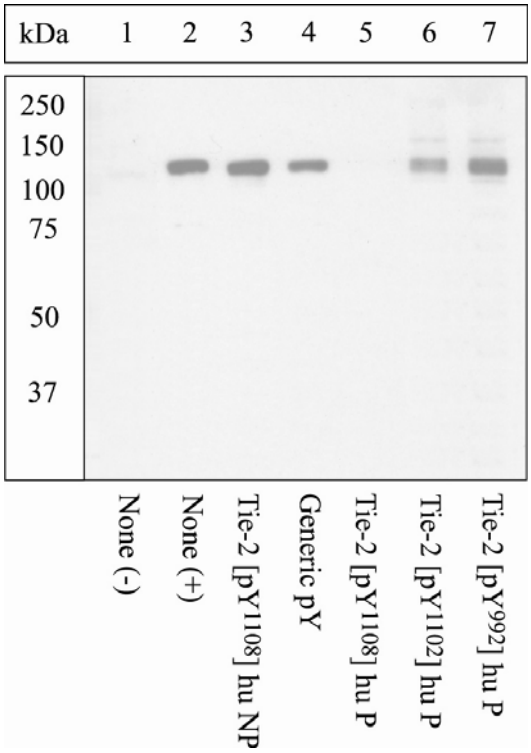

Antibody Specificity

Lysates prepared from HUVAC cells left alone (1) or allowed to grow on fibronectin-coated plates for 90 min (2-7) were resolved on a 10% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 3% BSA-TBST buffer for one hour at room temperature, and then incubated with the Tie-2 [pY^{1108}] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3) a generic phosphotyrosine-containing peptide (4) or the phosphopeptide immunogen corresponding to Tie2 [pY^{1108}] (5), a phosphopeptide immunogen corresponding to Tie2 [pY^{1102}] (6) or phosphopeptide corresponding to Tie2 [pY^{992}] (7). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ reagent.

The data show that the signal was induced by fibronectin and selectively blocked by the phosphopeptide corresponding to Tie2 [pY^{1108}] indicating that the signal is phosphorylation site-specific.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for one hour at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer
Formulation:
100 mL Tris buffered saline
3 gm Ig-free BSA
0.1 mL Tween 20

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