abcam

Product datasheet

Anti-TIE2 antibody [EPR21915] ab221154

Recombinant RabMAb

3 Images

Overview

Product name	Anti-TIE2 antibody [EPR21915]
Description	Rabbit monoclonal [EPR21915] to TIE2
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment within Human TIE2 aa 800-1100. The exact sequence is proprietary. Database link: Q02763
Positive control	WB: Human lung lysate; HUVEC and U-87 MG whole cell lysates. ICC/IF: HUVEC cells. Flow cyt: HUVEC cells.
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR21915
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab221154** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

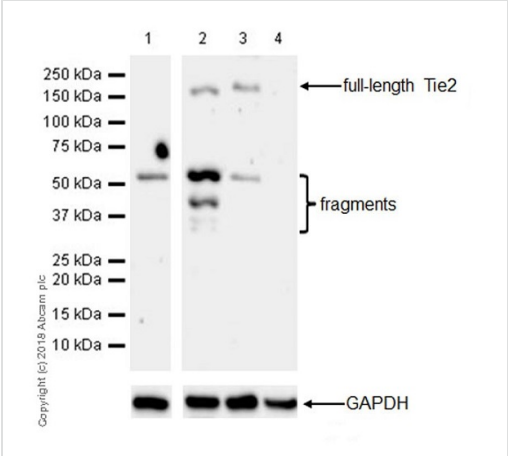
Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 160, 50, 40 kDa (predicted molecular weight: 126 kDa).
ICC/IF		1/4000.
Flow Cyt		1/500.

Target

Function	Tyrosine-protein kinase that acts as cell-surface receptor for ANGPT1, ANGPT2 and ANGPT4 and regulates angiogenesis, endothelial cell survival, proliferation, migration, adhesion and cell spreading, reorganization of the actin cytoskeleton, but also maintenance of vascular quiescence. Has anti-inflammatory effects by preventing the leakage of proinflammatory plasma proteins and leukocytes from blood vessels. Required for normal angiogenesis and heart development during embryogenesis. Required for post-natal hematopoiesis. After birth, activates or inhibits angiogenesis, depending on the context. Inhibits angiogenesis and promotes vascular stability in quiescent vessels, where endothelial cells have tight contacts. In quiescent vessels, ANGPT1 oligomers recruit TEK to cell-cell contacts, forming complexes with TEK molecules from adjoining cells, and this leads to preferential activation of phosphatidylinositol 3-kinase and the AKT1 signaling cascades. In migrating endothelial cells that lack cell-cell adhesions, ANGT1 recruits TEK to contacts with the extracellular matrix, leading to the formation of focal adhesion complexes, activation of PTK2/FAK and of the downstream kinases MAPK1/ERK2 and MAPK3/ERK1, and ultimately to the stimulation of sprouting angiogenesis. ANGPT1 signaling triggers receptor dimerization and autophosphorylation at specific tyrosine residues that then serve as binding sites for scaffold proteins and effectors. Signaling is modulated by ANGPT2 that has lower affinity for TEK, can promote TEK autophosphorylation in the absence of ANGPT1, but inhibits ANGPT1-mediated signaling by competing for the same binding site. Signaling is also modulated by formation of heterodimers with TIE1, and by proteolytic processing that gives rise to a soluble TEK extracellular domain. The soluble extracellular domain modulates signaling by functioning as decoy receptor for angiopoietins. TEK phosphorylates DOK2, GRB7, GRB14, PIK3R1; SHC1 and TIE1.
Tissue specificity	Detected in umbilical vein endothelial cells. Proteolytic processing gives rise to a soluble extracellular domain that is detected in blood plasma (at protein level). Predominantly expressed in endothelial cells and their progenitors, the angioblasts. Has been directly found in placenta and lung, with a lower level in umbilical vein endothelial cells, brain and kidney.
Involvement in disease	Dominantly inherited venous malformations May play a role in a range of diseases with a vascular component, including neovascularization of tumors, psoriasis and inflammation.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. Tie subfamily. Contains 3 EGF-like domains. Contains 3 fibronectin type-III domains. Contains 2 Ig-like C2-type (immunoglobulin-like) domains. Contains 1 protein kinase domain.
Domain	The soluble extracellular domain is functionally active in angiopoietin binding and can modulate the activity of the membrane-bound form by competing for angiopoietins.
Post-translational	Proteolytic processing leads to the shedding of the extracellular domain (soluble TIE-2 alias sTIE-

modifications	2). Autophosphorylated on tyrosine residues in response to ligand binding. Autophosphorylation occurs in trans, i.e. one subunit of the dimeric receptor phosphorylates tyrosine residues on the other subunit. Autophosphorylation occurs in a sequential manner, where Tyr-992 in the kinase activation loop is phosphorylated first, followed by autophosphorylation at Tyr-1108 and at additional tyrosine residues. ANGPT1-induced phosphorylation is impaired during hypoxia, due to increased expression of ANGPT2. Phosphorylation is important for interaction with GRB14, PIK3R1 and PTPN11. Phosphorylation at Tyr-1102 is important for interaction with SHC1, GRB2 and GRB7. Phosphorylation at Tyr-1108 is important for interaction with DOK2 and for coupling to downstream signal transduction pathways in endothelial cells. Dephosphorylated by PTPRB. Ubiquitinated. The phosphorylated receptor is ubiquitinated and internalized, leading to its degradation.
Cellular localization	Cell membrane. Cell junction. Cell junction, focal adhesion. Cytoplasm, cytoskeleton. Secreted. Recruited to cell-cell contacts in quiescent endothelial cells. Colocalizes with the actin cytoskeleton and at actin stress fibers during cell spreading. Recruited to the lower surface of migrating cells, especially the rear end of the cell. Proteolytic processing gives rise to a soluble extracellular domain that is secreted.

Images



Western blot - Anti-TIE2 antibody [EPR21915] (ab221154)

All lanes : Anti-TIE2 antibody [EPR21915] (ab221154) at 1/1000 dilution

Lane 1 : Human lung tissue lysate
Lane 2 : HUVEC (human umbilical vein endothelial cell line) whole cell lysate
Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate
Lane 4 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
Lane 1 : VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution
Lanes 2-4 : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 126 kDa
Observed band size: 160,40,50 kDa
why is the actual band size different from the predicted?

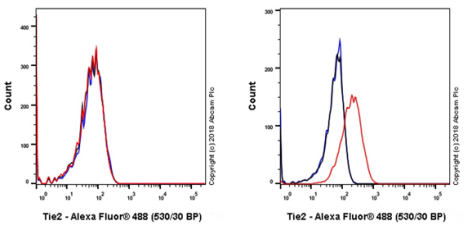
Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

The molecular mass observed is consistent with what has been described in the literature (PMID: 26666854). The antibody detects multiple bands (≤ 50 kDa) which are likely to be cleavage fragments of Tie2.

Negative control: HEK-293T (PMID: 15851516).

This blot was developed using a high sensitivity ECL substrate.

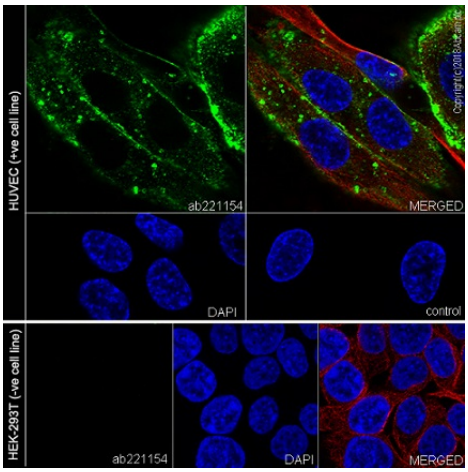


Flow Cytometry - Anti-TIE2 antibody [EPR21915] (ab221154)

Flow cytometric analysis of HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) (left panel) and HUVEC (human umbilical vein endothelial cell line) (right panel) cells labeling TIE2 with ab221154 at 1/500 dilution (red) compared with a Rabbit monoclonal IgG (ab172730) Isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077), at 1/2000 dilution was used as the secondary antibody.

Gated on viable cells.

Negative control: HEK-293T (PMID: 17189382)



Immunocytochemistry/ Immunofluorescence - Anti-TIE2 antibody [EPR21915] (ab221154)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HUVEC (human umbilical vein endothelial cell line) cells labeling TIE2 with ab221154 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous and cytoplasmic staining in HUVEC cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

Negative control: HEK-293T (PMID: 17189382).

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