Anti-TIE2 antibody [Cl. 16] ab24859

Overview

Product name
Anti-TIE2 antibody [Cl. 16]

Description
Mouse monoclonal [Cl. 16] to TIE2

Host species
Mouse

Tested applications
Suitable for: IHC-Fr, IHC-FoFr, WB, ELISA, Flow Cyt, IHC-P

Species reactivity
Reacts with: Mouse, Human

Immunogen
Recombinant fragment corresponding to Human TIE2. Recombinant human soluble extracellular TIE2.
Database link: Q02763

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.40
Constituent: PBS

Purity
Protein G purified

Clonality
Monoclonal

Clone number
Cl. 16

Isotype
IgG1

Applications

Our Abpromise guarantee covers the use of ab24859 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td>★★★★★</td>
<td>1/25. PubMed: 19253934</td>
</tr>
<tr>
<td>IHC-FoFr</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>
**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, ANGPT1 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.

### Application

<table>
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<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐️</td>
<td>Use a concentration of 1 - 2 µg/ml. Predicted molecular weight: 126 kDa.</td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td>Use a concentration of 1 - 2 µg/ml.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use 1-2µg for 10^6 cells. We tested in-house with methanol-fixed cells, but this may not be necessary since the antibody recognizes an extracellular epitope. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 20622121</td>
</tr>
</tbody>
</table>
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 modifications**
Proteolytic processing leads to the shedding of the extracellular domain (soluble TIE-2 alias sTIE-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**

**Images**

ab24859 staining TIE2 in C57 Mouse melanoma tumour tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde, permeabilized with 0.5% Tween 20 and blocked with 3% BSA for 1 hour at 23°C; antigen retrieval was by heat mediation in a Tris/HCl buffer. Samples were incubated with primary antibody (1/25 in 1% BSA, 0.5% Tween 20 and 1% Donkey serum) for 9 hours at 4°C. An Alexa Fluor® 555-conjugated Donkey polyclonal (1/1000) was used as the secondary antibody.

This image is courtesy of an Abreview submitted by Javier Rodriguez.
Western blot - Anti-TIE2 antibody [Cl. 16] (ab24859)

Immunohistochemistry (Frozen sections) - Anti-TIE2 antibody [Cl. 16] (ab24859)

ab24859 staining TIE2 in Human spleen by Immunohistochemistry (Frozen sections).
Immunohistochemistry (Frozen sections) - Anti-TIE2 antibody [Cl. 16] (ab24859)

This image is courtesy of an anonymous Abreview.

Immunohistochemical analysis of Human brain tissue, staining TIE2 with ab24859.

Tissue was fixed with paraformaldehyde, permeabilized with 0.25 Triton X-100 and blocked with 2.5% BSA for 30 minutes at 25°C. Samples were incubated with primary antibody (1/200 in 2.5% horse serum) for 18 hours at 4°C. An HRP-conjugated horse anti-rabbit polyclonal IgG was used as the secondary antibody.

ab24859 staining TIE2 in Human arterial wall tissue sections by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were fixed by perfusion with paraformaldehyde and blocked with 3% serum for 30 minutes at 25°C. Tissues were dunked in formalin for 72 hours before being embedded in OCT and sectioned. The sample was incubated with primary antibody (1/100 in 3% horse serum) at 4°C for 15 hours. A HRP-conjugated Horse vector impress was used as the secondary antibody.

Flow Cytometry - Anti-TIE2 antibody [Cl. 16] (ab24859)

Overlay histogram showing JEG-3 cells stained with ab24859 (red line). The cells were fixed with methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab24859, 2µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in JEG-3 cells fixed with 4% paraformaldehyde (10 min) used under the same conditions.

Please note that Abcam does not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.
All lanes: Anti-TIE2 antibody [Cl. 16] (ab24859)

Lane 1: HUVECs left untreated
Lane 2: HUVECs stimulated for 3 hours with PMA at 25 ng/ml
Lane 3: HUVECs stimulated for 6 hours with PMA at 25 ng/ml
Lane 4: HUVECs stimulated for 9 hours with PMA at 25 ng/ml
Lane 5: HUVECs stimulated for 24 hours with PMA at 25 ng/ml

Predicted band size: 126 kDa

Samples were immunoprecipitated with another TIE2 antibody.

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