abcam

ab221154

Anti-TIE2 antibody [EPR21915]

Anti-TIE2 antibody [EPR21915] (ab221154) is a rabbit monoclonal antibody detecting TIE2 in **Western Blot, Flow Cytometry (Intra), Flow Cytometry, ICC/IF, ELISA**. Suitable for **Human, Mouse**.

- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR21915
Purification technique	Affinity purification Protein A
Specificity	ab221154 shows low sensitivity in detecting full length of TIE2, we recommend using ab307556 or ab259982 as alternatives for detecting of full length of TIE2. TIE2 undergoes proteolytic cleavage (PMID: 32838837, PMID: 26666854). ab221154 is more sensitive in detecting C-terminal fragment at 60kDa.
Concentration	0.516 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/2500
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

I-ELISA

Tested

Species	Human
Dilution info	0.25 µg/mL
Notes	-

Species	Mouse
Dilution info	0.25 µg/mL
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/4000
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information TEK

Function	Tyrosine-protein kinase that acts as a 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 pro-inflammatory 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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-TIE2 antibody [EPR21915] (ab221154) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunocytochemistry/immunofluorescence (ICC/IF), ELISA in Human, Mouse samples.

What is the molecular weight of TIE2?
Anti-TIE2 [EPR21915] (ab221154) specifically detects a band for TIE2 (UniProt: Q02763) at a molecular weight of 126kDa.

Other related products

We have a range of other formats of antibody clone [EPR21915] also available for your convenience: ab221154, Carrier free - ab238172, Alexa Fluor® 647 - ab306575, Alexa Fluor® 488 - ab307374, PE - ab307642, APC - ab314268

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	TIE2 also known as TEK or CD202B is a receptor tyrosine kinase with a mass of approximately 145 kDa. It plays an important role in vascular development and maintenance. TIE2 is mainly expressed in endothelial cells which line the interior surface of blood vessels. It binds angiopoietins predominantly Angiopoietin-1 (Angpt1) influencing vascular stability and permeability. Researchers use TIE2 antibodies including anti-TIE and anti-clone to study its role and functions across various contexts.
Biological function summary	TIE2 plays a central role in angiogenesis and blood vessel maturation. It forms a receptor complex with other components to mediate its effects. This interaction mainly involves Angpt1 which binds to TIE2 stabilizing blood vessels and preserving their integrity. TIE2 also participates in cell survival and migration processes necessary for normal vascular function. Researchers often perform Angpt1 ELISA in bulk to quantify interactions involving TIE2 in experiments.
Pathways	TIE2 is important in the angiopoietin-TIE signaling pathway regulating vascular development. TIE2 is related to proteins such as Angpt1 which are centrally involved in this pathway. It also interacts with various signaling molecules including phosphatidylinositol-3-kinase (PI3K) which contributes to cell survival pathways. Alterations in the TIE2 signaling cascade can lead to aberrant angiogenesis and related pathologies.
Associated diseases and disorders	TIE2 is implicated in several vascular diseases and cancer. Mutations or dysregulation of TIE2 can result in venous malformations and further compromise blood vessel integrity. In cancer TIE2 expression can influence tumor angiogenesis enhancing tumor growth and metastasis. TIE2 interacts with proteins like vascular endothelial growth factor (VEGF) which are involved in neovascularization and

cancer progression. Understanding these interactions helps target therapeutic strategies in relevant diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

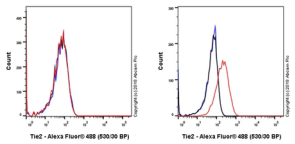
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

10 product images

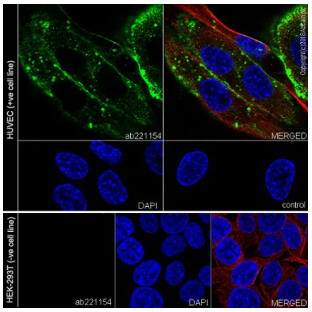


Flow Cytometry - Anti-TIE2 antibody [EPR21915] (ab221154)

TIE2 Flow Cytometry staining using rabbit Anti-TIE2 antibody

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)

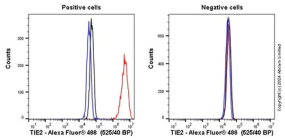


Immunocytochemistry/ Immunofluorescence - Anti-TIE2 antibody [EPR21915] (ab221154)

TIE2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-TIE2 antibody

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).



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

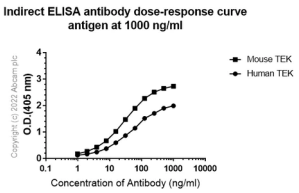
TIE2 Flow Cytometry (Intracellular) staining using rabbit Anti-TIE2 antibody

Flow cytometry overlay histogram showing positive HUVEC cells (left) and negative Hek-293 cells (right) stained with ab221154 (red line). The cells were fixed with 4% PFA and permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab221154) (1x 10^6 in 100µl at 0.2 µg/ml (1/2,500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C.

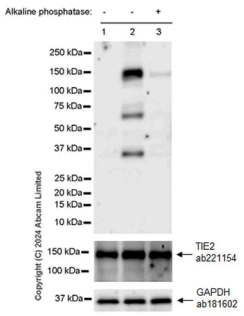
Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (black line). Unlabelled sample was also used as a control (blue line).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Indirect ELISA - Anti-TIE2 antibody [EPR21915] (ab221154)

Indirect ELISA showing primary antibody ab221154 binding to mouse TEK and Human TEK. Antigen concentration is 1000 ng/ml. Binding of ab221154 was assessed in a serial dilution range 1000-0 ng/ml. Binding was detected using the secondary antibody, Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution.



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

TIE2 Western blot staining using rabbit Anti-TIE2 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The identity of the bands lower than 100 kDa are unknown.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-TIE2 antibody [EPR21915] - (ab221154) staining at 1/500 dilution.

All lanes:
Western blot - Anti-TIE2 (phospho Y992) antibody [EPR27958-1] (ab321883) at 1/1000 dilution

Lane 1:
Untreated HUVEC (human umbilical vein endothelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
HUVEC treated with 100 uM Pervanadate for 10 minutes whole cell lysate (untreated membrane) at 20 µg

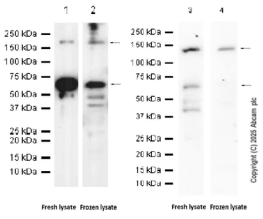
Lane 3:
HUVEC treated with 100 uM Pervanadate for 10 minutes whole cell lysate (alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 36 kDa, 126 kDa

Exposure time: 103s



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

TIE2 Western blot staining using rabbit Anti-TIE2 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

To preserve protein degradation, harvested cells were immediately lysed, followed by rapid gel electrophoresis and membrane transfer procedures.

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

Lane 1:
HUVEC (human umbilical vein endothelial cell line) fresh whole cell lysate at 20 µg

Lane 2:
HUVEC (human umbilical vein endothelial cell line) frozen whole cell lysate at 20 µg

Lane 3:
Mouse lung fresh tissue lysate at 20 µg

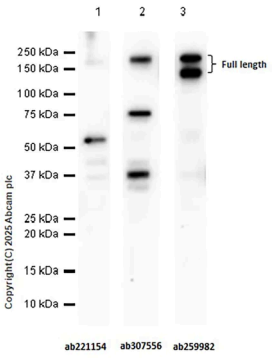
Lane 4:
Mouse lung frozen tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 160 kDa, 60 kDa

Exposure time: 180s



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

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The antibody ab221154 exhibits low sensitivity in Western blot analyses. To improve detection of full-length TIE2, we recommend substituting with ab307556 or ab259982. Alternatively, experimental conditions can be optimized through increasing sample loading quantity, using lower antibody dilution or implementing femtogram-level sensitivity detection substrates.

Important note: TIE2 undergoes proteolytic cleavage (PMID: 32838837, PMID: 26666854). These three antibodies target distinct cleaved fragments of TIE2 due to differences in their immunogen localization.

Lane 1:
Western blot - Anti-TIE2 antibody [EPR21915] (ab221154)

Lane 2:
Western blot - Anti-TIE2 antibody [EPR26540-78] (ab307556)

Lane 3:
Western blot - Anti-TIE2 antibody [EPR23823-109] (ab259982)

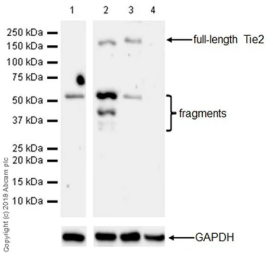
All lanes:
HUVEC (human umbilical vein endothelial cell line) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 60 kDa, 40 kDa, 160 kDa

Exposure time: 40s



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

TIE2 Western blot staining using rabbit Anti-TIE2 antibody

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. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-TIE2 antibody [EPR21915] (ab221154) at 1/100000 dilution

Lane 1:
Human lung tissue lysate at 20 µg

Lane 2:
HUVEC (human umbilical vein endothelial cell line) whole cell lysate at 20 µg

Lane 3:
U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate at 20 µg

Lane 4:
HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate at 20 µg

Secondary

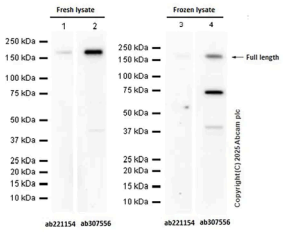
All lanes:
Western blot - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

Developed using the ECL technique.

Predicted band size: 125 kDa

Observed band size: 40 kDa, 50 kDa, 160 kDa

Exposure time: 3min



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

TIE2 Western blot staining using rabbit Anti-TIE2 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The antibody ab221154 exhibits low sensitivity in Western blot analyses. To improve detection of full-length TIE2 in mouse samples, we recommend substituting with ab307556. Alternatively, experimental conditions can be optimized through increasing sample loading quantity, using lower antibody dilution or implementing femtogram-level sensitivity detection substrates.

To preserve protein degradation, harvested cells were immediately lysed, followed by rapid gel electrophoresis and membrane transfer procedures.

Lanes 1 and 3:
Western blot - Anti-TIE2 antibody [EPR21915] (ab221154) at 1/1000 dilution

Lanes 2 and 4:
Western blot - Anti-TIE2 antibody [EPR26540-78] (ab307556) at 1/1000 dilution

Lanes 1 - 2:
bEnd.3 (mouse brain endothelial cell) fresh whole cell lysate at 20 µg

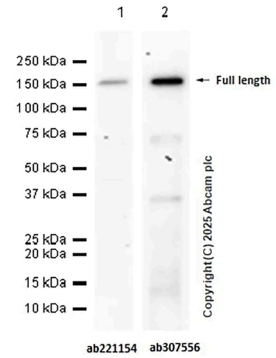
Lanes 3 - 4:
bEnd.3 (mouse brain endothelial cell) frozen whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 160 kDa

Exposure time: 180s



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

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The antibody ab221154 exhibits low sensitivity in Western blot analyses. To improve detection of full-length TIE2 in mouse samples, we recommend substituting with ab307556. Alternatively, experimental conditions can be optimized through increasing sample loading quantity, using lower antibody dilution or implementing femtogram-level sensitivity detection substrates.

Lane 1:
Western blot - Anti-TIE2 antibody [EPR21915] (ab221154) at 1/1000 dilution

Lane 2:
Western blot - Anti-TIE2 antibody [EPR26540-78] (ab307556) at 1/1000 dilution

All lanes:
Mouse lung tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 160 kDa

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.