abcam

Product datasheet

Anti-Eph receptor B4/HTK antibody [EPR23221-54] - BSA and Azide free ab269867





4 Images

Overview

Product name Anti-Eph receptor B4/HTK antibody [EPR23221-54] - BSA and Azide free

Rabbit monoclonal [EPR23221-54] to Eph receptor B4/HTK - BSA and Azide free **Description**

Host species Rabbit

Suitable for: IP, WB **Tested applications**

Unsuitable for: Flow Cyt,ICC/IF or IHC-P

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK293T, HT-29 and MCF7 lysates. IP: HT-29 and HUVEC whole cell lysates.

General notes ab269867 is the carrier-free version of ab254300.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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 production

For more information see here.

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

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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR23221-54

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab269867 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
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 108 kDa.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IHC-P.

Target

Function Receptor for members of the ephrin-B family. Binds to ephrin-B2. May have a role in events

mediating differentiation and development.

Tissue specificity Abundantly expressed in placenta and in a range of primary tissues and malignant cell lines.

Expressed in fetal, but not adult, brain, and in primitive and myeloid, but not lymphoid,

hematopoietic cells.

Sequence similaritiesBelongs to the protein kinase superfamily. Tyr protein kinase family. Ephrin receptor subfamily.

Contains 2 fibronectin type-III domains. Contains 1 protein kinase domain.

Contains 1 SAM (sterile alpha motif) domain.

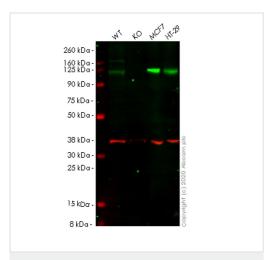
Post-translational

modifications

 $\label{eq:Autophosphorylated} Autophosphorylated.$

Cellular localization Membrane.

Images



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23221-54] - BSA and Azide free (ab269867)

All lanes : Anti-Eph receptor B4/HTK antibody [EPR23221-54] (ab254300) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: EPHB4 knockout HEK293T cell lysate

Lane 3 : MCF7 cell lysate

Lane 4 : HT-29 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

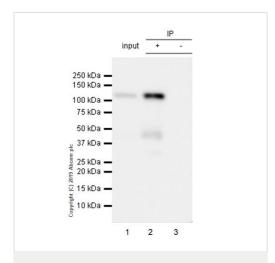
All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 108 kDa **Observed band size:** 125 kDa

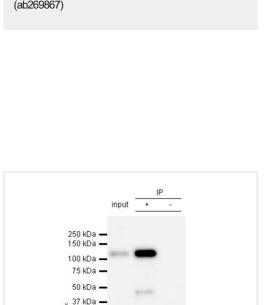
This data was developed using the same antibody clone in a different buffer formulation (ab254300).

Lanes 1-4: Merged signal (red and green). Green - <u>ab254300</u> observed at 125 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab254300 Anti-Eph receptor B4/HTK antibody [EPR23221-54] was shown to specifically react with Eph receptor B4/HTK in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266733 (knockout cell lysate ab257429) was used. Wild-type and Eph receptor B4/HTK knockout samples were subjected to SDS-PAGE. ab254300 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-Eph receptor B4/HTK antibody [EPR23221-54] - BSA and Azide free (ab269867)



Immunoprecipitation - Anti-Eph receptor B4/HTK antibody [EPR23221-54] - BSA and Azide free (ab269867)

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25 kDa -

20 kDa -

2 15 kDa —

Eph receptor B4 was immunoprecipitated from 0.35 mg HUVEC (human umbilical vein endothelial cell) whole cell lysate with ab254300 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254300 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/1000 dilution.

Lane 1: HUVEC (human umbilical vein endothelial cell) whole cell lysate 10ug

Lane 2: ab254300 IP in HUVEC whole cell lysate

Lane 3: Rabbit monoclonal $\lg G \left(\underline{ab172730} \right)$ instead of $\underline{ab254300}$ in HUVEC whole cell lysate

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

Exposure time: 8 seconds.

Cleaved intracellular domain (ICD) was observed at around 47kDa. (PMID: 24854540).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254300).

Eph receptor B4 was immunoprecipitated from 0.35 mg HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate with <u>ab254300</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab254300</u> at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used at 1/1000 dilution.

Lane 1: HT-29 (human colorectal adenocarcinoma epithelial cell) whole cell lysate 10ug

Lane 2: ab254300 IP in HT-29 whole cell lysate

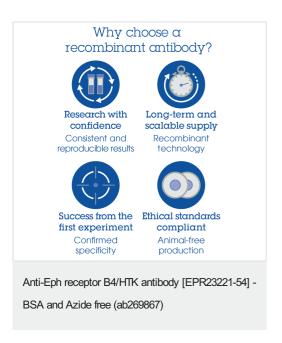
Lane 3: Rabbit monoclonal IgG ($\underline{ab172730}$) instead of $\underline{ab254300}$ in HT-29 whole cell lysate

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

Exposure time: 8 seconds.

Cleaved intracellular domain (ICD) was observed at around 47kDa. (PMID: 24854540).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254300).



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