

Product datasheet

Anti-Eph receptor B4/HTK antibody [EPR23222-24] - BSA and Azide free ab269369

KO VALIDATED Recombinant RabMAb

5 Images

Overview

Product name	Anti-Eph receptor B4/HTK antibody [EPR23222-24] - BSA and Azide free
Description	Rabbit monoclonal [EPR23222-24] to Eph receptor B4/HTK - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: IHC-P, WB Unsuitable for: Flow Cyt, ICC/IF or IP
Species reactivity	Reacts with: Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293T, HT-29, HUVEC, HCT116, MCF7, T47D, PC-3, NIH:OVCAR-3, SK-BR-3, GR-M, 4T1 and NIH/3T3 lysates. IHC-P: Human colon, Human colon cancer and Human breast cancer tissues.
General notes	<p>ab269369 is the carrier-free version of ab254301.</p> <p>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.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>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.</p> <p>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.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit</p>

monoclonal antibodies. For details on our patents, please refer to [RabMAb® patents](#).

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
Clonality	Monoclonal
Clone number	EPR23222-24
Isotype	IgG

Applications

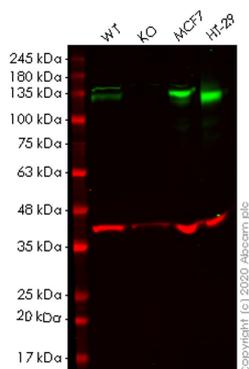
The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab269369 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 108 kDa).

Application notes Is unsuitable for Flow Cyt, ICC/IF or IP.

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 similarities	Belongs 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	Autophosphorylated.
Cellular localization	Membrane.



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23222-24] - BSA and Azide free (ab269369)

All lanes : Anti-Eph receptor B4/HTK antibody [EPR23222-24] ([ab254301](#)) 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 IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

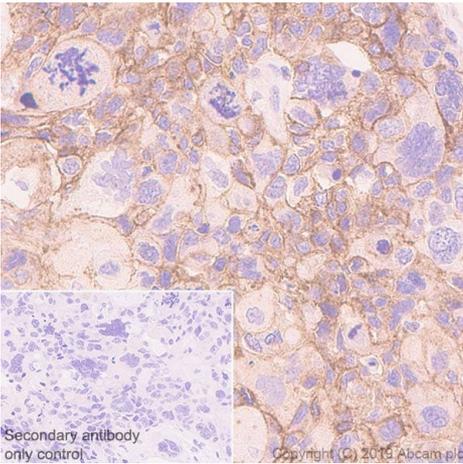
Predicted band size: 108 kDa

Observed band size: 125 kDa

This data was developed using the same antibody clone in a different buffer formulation ([ab254301](#)).

Lanes 1-4: Merged signal (red and green). Green - [ab254301](#) observed at 125 kDa. Red - loading control [ab8245](#) observed at 36 kDa.

[ab254301](#) Anti-Eph receptor B4/HTK antibody [EPR23222-24] 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. [ab254301](#) 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 IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



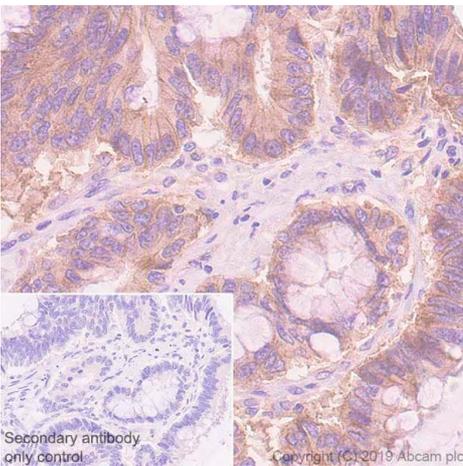
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Eph receptor B4/HTK antibody [EPR23222-24] - BSA and Azide free (ab269369)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling Eph receptor B4 with [ab254301](#) at 1/1000 dilution (0.545ug/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) at a ready to use dilution. Mainly membranous staining in cancer cells of human breast cancer (PMID: 16816380) is observed. The section was incubated with [ab255611](#) for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) at Ready to use dilution.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab254301](#)).



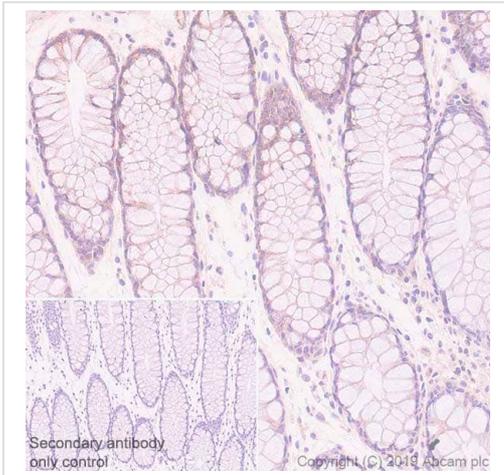
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Eph receptor B4/HTK antibody [EPR23222-24] - BSA and Azide free (ab269369)

Immunohistochemical analysis of paraffin-embedded Human colon cancer tissue labeling Eph receptor B4 with [ab254301](#) at 1/1000 dilution (0.545ug/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) at a ready to use dilution. Positive staining in cancer cells of human colon cancer (PMID: 19366806) is observed. The section was incubated with [ab255611](#) for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) at Ready to use dilution.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab254301](#)).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Eph receptor B4/HTK antibody [EPR23222-24] - BSA and Azide free (ab269369)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Eph receptor B4 with [ab254301](#) at 1/1000 dilution (0.545ug/ml) followed by a Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) at a ready to use dilution. Weak positive staining in base of crypts of human colon (PMID:19366806) is observed. The section was incubated with [ab255611](#) for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) at Ready to use dilution.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab254301](#)).

Why choose a recombinant antibody?

 Research with confidence Consistent and reproducible results	 Long-term and scalable supply Recombinant technology
 Success from the first experiment Confirmed specificity	 Ethical standards compliant Animal-free production

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Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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