

Product datasheet

Anti-Eph receptor B4/HTK antibody [EPR23222-24] ab254301

KO VALIDATED Recombinant RabMAb[®]

[1 References](#) [7 Images](#)

Overview

Product name	Anti-Eph receptor B4/HTK antibody [EPR23222-24]
Description	Rabbit monoclonal [EPR23222-24] to Eph receptor B4/HTK
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P 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>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 monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR23222-24
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab254301 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 120 kDa (predicted molecular weight: 108 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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.

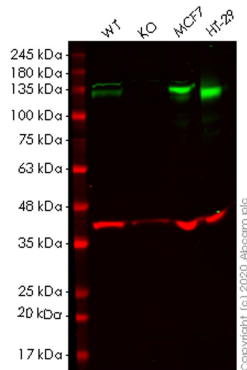
Tissue specificity Abundantly expressed in placenta and in a range of primary tissues and malignant cell lines. Expressed in fetal, but not adult, brain, and in primitive and myeloid, but not lymphoid, hematopoietic cells.

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.

Images



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301)

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

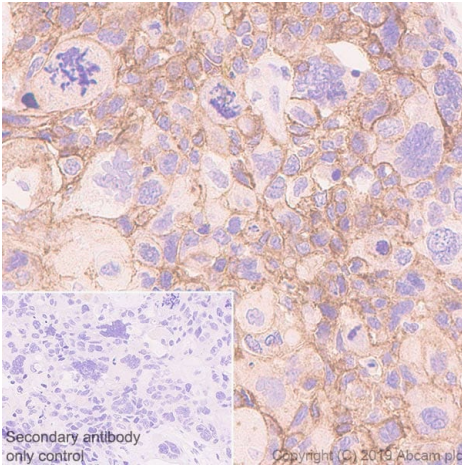
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 108 kDa

Observed band size: 125 kDa

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.

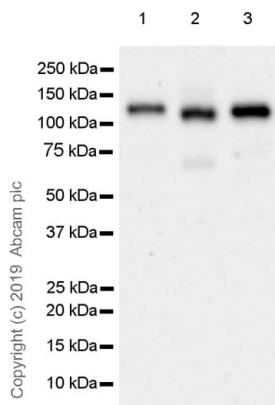


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301)

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 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.



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301)

All lanes : Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301) at 1/1000 dilution

Lane 1 : GR-M (mouse Caucasian melanoma epithelial cell), whole cell lysate

Lane 2 : 4T1 (mouse mammary gland carcinoma epithelial cell), whole cell lysate

Lane 3 : NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : MGMT overexpression 293T lysate (whole cell) (**ab94051**) at 1/100000 dilution

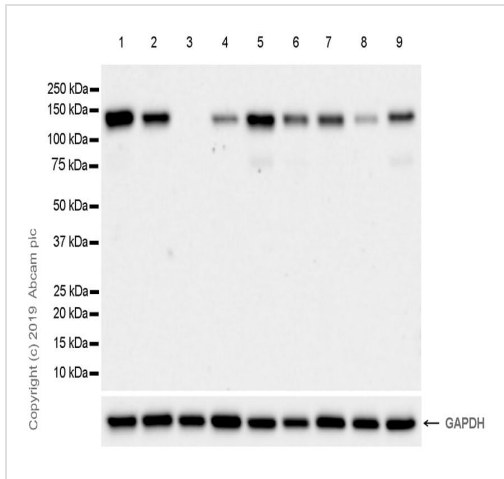
Predicted band size: 108 kDa

Observed band size: 120 kDa

Blocking and dilution buffer: 5% NFD/MTBST.

Exposure time: 92 seconds.

The expression profile observed is consistent with what has been described in the literature (PMID: 19366806, 16840724).



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301)

All lanes : Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301) at 1/1000 dilution

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

Lane 2 : HUVEC (human umbilical vein endothelial cell), whole cell lysate

Lane 3 : SW620 (human colorectal adenocarcinoma epithelial cell), whole cell lysate

Lane 4 : HCT116 (human colorectal carcinoma epithelial cell), whole cell lysate

Lane 5 : MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lane 6 : T47D (human ductal breast epithelial tumor epithelial cell), whole cell lysate

Lane 7 : PC-3 (human prostate adenocarcinoma epithelial cell), whole cell lysate

Lane 8 : NIH:OVCAR-3 (human ovary adenocarcinoma epithelial cell), whole cell lysate

Lane 9 : SK-BR-3 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 108 kDa

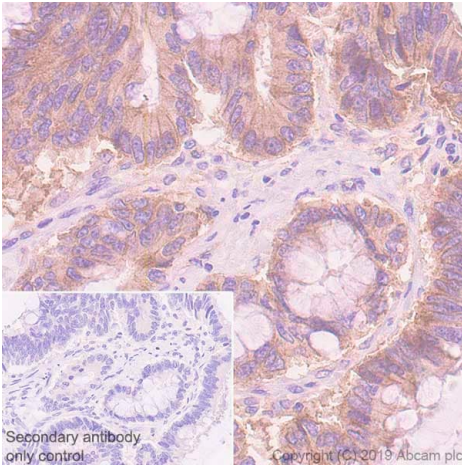
Observed band size: 120 kDa

Blocking and dilution buffer: 5% NFD/MTBST.

Exposure time: 70 seconds.

Negative control: SW620 (PMID: 19366806).

EphB4 is negative in SW620 and positive in HT29 and HUVEC, which is different from the expression profiles of other EphB family members. The expression profile observed is consistent with what has been described in the literature (PMID: 19366806, 16840724).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301)

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Eph receptor B4/HTK antibody [EPR23222-24] (ab254301)

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.

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

Anti-Eph receptor B4/HTK antibody [EPR23222-24]
(ab254301)

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

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