

## Product datasheet

# Anti-Eph receptor B4/HTK antibody [EPR23221-54] ab254300

**KO VALIDATED** Recombinant RabMAb<sup>®</sup>

[5 Images](#)

### Overview

<b>Product name</b>	Anti-Eph receptor B4/HTK antibody [EPR23221-54]
<b>Description</b>	Rabbit monoclonal [EPR23221-54] to Eph receptor B4/HTK
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> WB, IP <b>Unsuitable for:</b> Flow Cyt, ICC/IF or IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: HEK293T, HT-29, HUVEC, HCT 116 and MCF7 lysates. IP: HT-29 and HUVEC whole cell lysates.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal

**Clone number**                      EPR23221-54

**Isotype**                                IgG

## Applications

---

**The Abpromise guarantee**            Our **Abpromise guarantee** covers the use of ab254300 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).
IP		1/30.

**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 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.

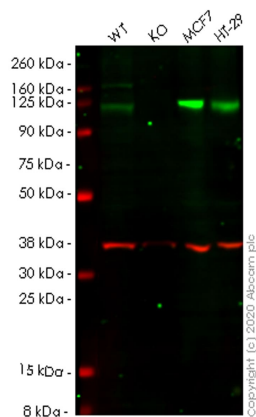
**Post-translational modifications**        Autophosphorylated.

**Cellular localization**                      Membrane.

---

## Images

---



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23221-54] (ab254300)

**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

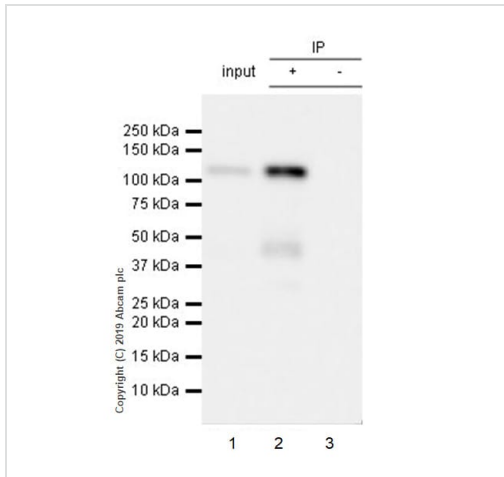
**All lanes** : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

**Predicted band size:** 108 kDa

**Observed band size:** 125 kDa

**Lanes 1-4:** Merged signal (red and green). Green - ab254300 observed at 125 kDa. Red - loading control **ab8245** 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 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.



Immunoprecipitation - Anti-Eph receptor B4/HTK antibody [EPR23221-54] (ab254300)

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

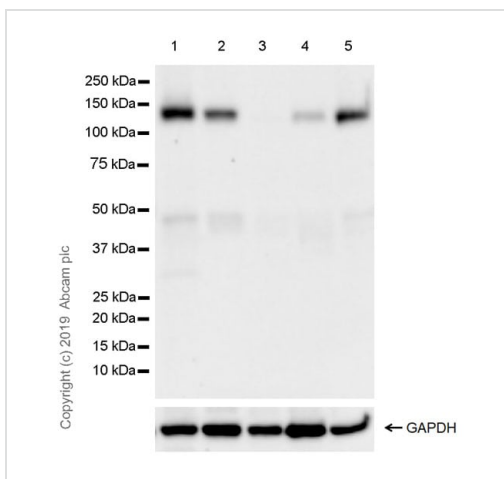
Lane 2: ab254300 IP in HUVEC whole cell lysate

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of 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).



Western blot - Anti-Eph receptor B4/HTK antibody [EPR23221-54] (ab254300)

**All lanes :** Anti-Eph receptor B4/HTK antibody [EPR23221-54] (ab254300) 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 :** HCT 116 (human colorectal carcinoma epithelial cell), whole cell lysate

**Lane 5 :** MCF7 (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% NFDm/TBST.

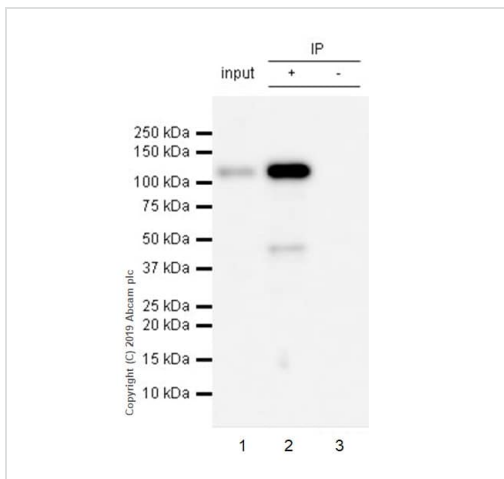
Exposure time: 37 seconds.

**Negative control:** SW620 (PMID: 19366806).

EphB4 is negative in SW620 and positive in HT29 and HUVEC, which is different from expression profiles of other EphB family members.

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

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



Immunoprecipitation - Anti-Eph receptor B4/HTK antibody [EPR23221-54] ([ab254300](#))

Eph receptor B4 was immunoprecipitated from 0.35 mg HT-29 (human colorectal adenocarcinoma epithelial 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: 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 ([ab172730](#)) instead of [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).

### 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 [EPR23221-54]  
(ab254300)

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

### Our Abpromise to you: Quality guaranteed and expert technical support

---

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

### Terms and conditions

---

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors