# abcam

## Product datasheet

# Anti-Eph receptor A2 antibody [EPR17660-120] ab185156



## 1 References 6 Images

#### Overview

Product name Anti-Eph receptor A2 antibody [EPR17660-120]

**Description** Rabbit monoclonal [EPR17660-120] to Eph receptor A2

Host species Rabbit

Tested applications Suitable for: WB, ICC/IF, IP, Flow Cyt

Species reactivity Reacts with: Mouse, Rat

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: NIH/3T3, L-929, F9 and C6 whole cell lysates. ICC/IF: NIH/3T3 cells. Flow Cyt: NIH/3T3 cells.

IP: NIH/3T3 whole cell lysate.

**General notes**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 supplyAnimal-free production

For more information see here.

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

#### **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: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS

Purity Protein A purified

**Clonality** Monoclonal

Clone number EPR17660-120

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**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab185156 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 130 kDa (predicted molecular weight: 109 kDa).
ICC/IF		1/50.
IP		1/30.
Flow Cyt		1/50.

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**Function** 

Receptor for members of the ephrin-A family. Binds to ephrin-A1, -A3, -A4 and -A5. Plays an important role in angiogenesis and tumor neovascularization. The recruitement of VAV2, VAV3 and Pl3-kinase p85 subunit by phosphorylated EPHA2 is critical for EFNA1-induced RAC1 GTPase activation and vascular endothelial cell migration and assembly (By similarity). Induces apoptosis in a p53/TP53-independent, caspase-8-dependent manner.

**Tissue specificity** 

Expressed in brain and glioma tissue and glioma cell lines (at protein level). Expressed most highly in tissues that contain a high proportion of epithelial cells, e.g., skin, intestine, lung, and ovary.

Involvement in disease

Genetic variations in EPHA2 are the cause of susceptibility to cataract cortical age-related type 2 (ARCC2) [MIM:613020]. A developmental punctate opacity common in the cortex and present in most lenses. The cataract is white or cerulean, increases in number with age, but rarely affects vision.

Defects in EPHA2 are the cause of cataract posterior polar type 1 (CTPP1) [MIM:116600]. A subcapsular opacity, usually disk-shaped, located at the back of the lens. It can have a marked effect on visual acuity.

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.

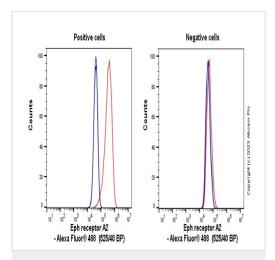
Contains 1 SAM (sterile alpha motif) domain.

Post-translational modifications

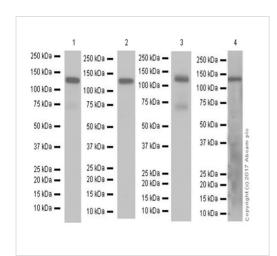
Activated by EFNA1 via tyrosine phosphorylation. Phosphorylated residues Tyr-588 and Tyr-594 are required for binding VAV2 and VAV3 while phosphorylated residues Tyr-735 and Tyr-930 are required for binding Pl3-kinase p85 subunit. These phosphorylated residues are critical for recruitment of VAV2 and VAV3 and Pl3-kinase p85 subunit which transduce downstream signaling to activate RAC1 GTPase and endothelial cell migration. They also play a critical role in transducing EPHA2 signaling in vascular endothelial cells during tumor angiogenesis.

**Cellular localization** 

Membrane.



Flow Cytometry - Anti-Eph receptor A2 antibody [EPR17660-120] (ab185156)



Western blot - Anti-Eph receptor A2 antibody [EPR17660-120] (ab185156)

Flow cytometry overlay histogram showing left NIH3T3 positive cells and right negative B16-F10 stained with ab185156 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interactionfollowed by the antibody (ab185156) (1x  $10^6$  in  $100\mu$ l at  $5.0~\mu$ g/ml (1/424)) for 30min on ice.

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

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

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

**All lanes :** Anti-Eph receptor A2 antibody [EPR17660-120] (ab185156) at 1/1000 dilution

**Lane 1**: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 2: L-929 (mouse connective tissue fibroblast cell line) whole cell lysate

Lane 3: F9 (mouse embryonic testicular cancer cell line) whole cell lysate

Lane 4: C6 (rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 109 kDa Observed band size: 130 kDa Blocking/Dilution buffer: 5% NFDM/TBST.

ab185156 MERGED

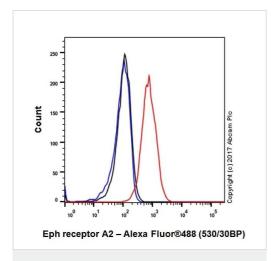
DAPI Secondary antibody only control

Immunocytochemistry/ Immunofluorescence - Anti-Eph receptor A2 antibody [EPR17660-120] (ab185156)

Immunofluorescent analysis of 100% methanol-fixed NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling Eph receptor A2 with ab185156 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining on NIH/3T3 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (**ab195889**) (red) at 1/200 dilution.

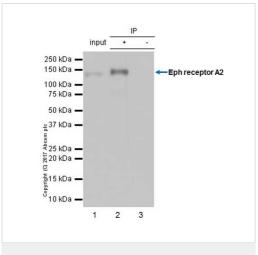
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.



Flow Cytometry - Anti-Eph receptor A2 antibody [EPR17660-120] (ab185156)

Flow cytometric analysis of NIH/3T3 (mouse embryonic fibroblast cell line) cell line labeling Eph receptor A2 with ab185156 at 1/50 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.

Total viable cells were gated for the FC image.



Immunoprecipitation - Anti-Eph receptor A2 antibody [EPR17660-120] (ab185156)

Eph receptor A2 was immunoprecipitated from 0.35 mg of NIH/3T3 (mouse embryonic fibroblast cell line) lysate with ab185156 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab185156 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

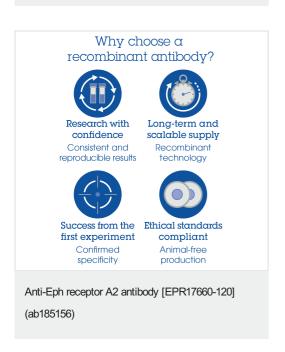
Lane 1: NIH/3T3 whole cell lysate 10 µg (Input).

Lane 2: ab185156 IP in NIH/3T3 whole cell lysate.

Lane 3: Rabbit monoclonal  $\lg G$  (ab172730) instead of ab185156 in NIH/3T3 whole cell lysate.

Exposure time: 10 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.



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