# abcam

#### Product datasheet

# Anti-VAV2 antibody [EP1067Y] ab52640





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#### Overview

**Product name** Anti-VAV2 antibody [EP1067Y]

**Description** Rabbit monoclonal [EP1067Y] to VAV2

**Host species** Rabbit

Specificity This antibody detects Vav2 phosphorylated on Tyr172 as well as unphosphorylated Vav2.

**Tested applications** Suitable for: Flow Cyt (Intra), WB, IP, IHC-P

Species reactivity Reacts with: Mouse, Human

Predicted to work with: Rat

**Immunogen** Synthetic peptide within Human VAV2. The exact sequence is proprietary.

Positive control WB: HeLa cells; HAP1 cells; HEK293 cells. IHC-P: Human cervical carcinoma. Flow Cyt (intra):

HeLa cells. IP: HEK293 cells.

**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 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**.

#### **Properties**

**Form** Liquid

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

**Purity** Protein A purified

Clonality Monoclonal

Clone number EP1067Y

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab52640 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
Flow Cyt (Intra)		1/2500.
WB	****(3)	1/20000. Detects a band of approximately 101 kDa (predicted molecular weight: 101 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

#### **Target**

**Function** Guanine nucleotide exchange factor for the Rho family of Ras-related GTPases. Plays an

important role in angiogenesis. Its recruitement by phosphorylated EPHA2 is critical for EFNA1-

induced RAC1 GTPase activation and vascular endothelial cell migration and assembly.

**Tissue specificity** Widely expressed.

Sequence similarities Contains 1 CH (calponin-homology) domain.

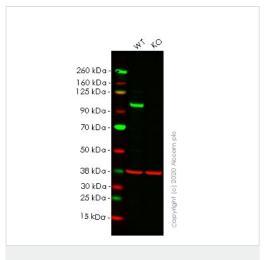
Contains 1 DH (DBL-homology) domain.

Contains 1 PH domain.

Contains 1 phorbol-ester/DAG-type zinc finger.

Contains 1 SH2 domain. Contains 2 SH3 domains.

#### **Images**



Western blot - Anti-VAV2 antibody [EP1067Y] (ab52640)

**All lanes :** Anti-VAV2 antibody [EP1067Y] (ab52640) at 1/20000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: VAV2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 101 kDa **Observed band size:** 100 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab52640 observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

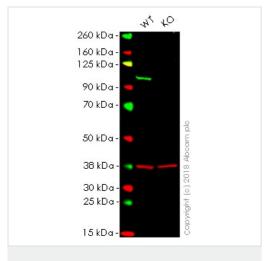
ab52640 was shown to react with VAV2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265318 (knockout cell lysate ab257794) was used. Wild-type HeLa and VAV2 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab52640 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 20000 dilution and a 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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-VAV2 antibody
[EP1067Y] (ab52640)

Human cervical carcinoma stained with ab52640 at 1/50 - 1/100 dilution

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-VAV2 antibody [EP1067Y] (ab52640)

**All lanes :** Anti-VAV2 antibody [EP1067Y] (ab52640) at 1/20000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: VAV2 knockout HAP1 whole cell lysate

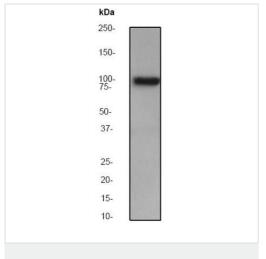
Lysates/proteins at 20 µg per lane.

Predicted band size: 101 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab52640 observed at 101 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab52640 was shown to specifically react with VAV2 in wild-type HAP1 cells as signal was lost in VAV2 knockout cells. Wild-type and VAV2 knockout samples were subjected to SDS-PAGE.

Ab52640 and ab9484 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/20000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



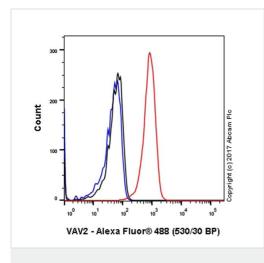
Western blot - Anti-VAV2 antibody [EP1067Y] (ab52640)

Anti-VAV2 antibody [EP1067Y] (ab52640) at 1/20000 dilution + 293 cell lysate at 10  $\mu g$ 

### Secondary

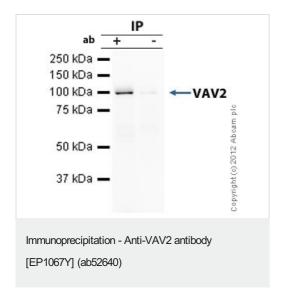
Goat anti-rabbit HRP labeled at 1/2000 dilution

Predicted band size: 101 kDa Observed band size: 101 kDa



Flow Cytometry (Intracellular) - Anti-VAV2 antibody [EP1067Y] (ab52640)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling VAV2 (red) with ab52640 at a 1/2500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit lgG (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal lgG (ab172730). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.

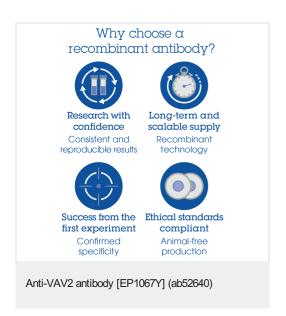


VAV2 was immunoprecipitated using 0.5mg Hek293 whole cell extract,  $10\mu g$  of Rabbit monoclonal to VAV2 and  $50\mu l$  of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hek293 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of  $40\mu l$  SDS loading buffer and incubated for 10min at  $70^{o}C$ ;  $10\mu l$  of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab52640.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

Band: 100kDa; VAV2.



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