

### Human VASP knockout HeLa cell line ab265892

3 Images

#### Overview

<b>Product name</b>	Human VASP knockout HeLa cell line
<b>Parental Cell Line</b>	HeLa
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, Homozygous: 13 bp deletion in exon 2
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing, Western Blot (WB)
<b>Tested applications</b>	<b>Suitable for:</b> WB
<b>Biosafety level</b>	2
<b>General notes</b>	<p><b>Recommended control:</b> Human wild-type HeLa cell line (<a href="#">ab255928</a>). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.</p> <p><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> DMEM (High Glucose) + 10% FBS</p> <p><b>Initial handling guidelines:</b> Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.</p> <ol style="list-style-type: none"> <li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.</li> <li>3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of <math>2 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of <math>2 \times 10^4</math> cells/cm<sup>2</sup> is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Cervix
<b>Cell type</b>	epithelial
<b>Disease</b>	Adenocarcinoma
<b>Gender</b>	Female
<b>STR Analysis</b>	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 WWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
<b>Antibiotic resistance</b>	Puromycin 1.00µg/ml
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

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<b>Function</b>	Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity such as axon guidance, lamellipodial and filopodial dynamics, platelet activation and cell migration. VASP promotes actin filament elongation. It protects the barbed end of growing actin filaments against capping and increases the rate of actin polymerization in the presence of capping protein. VASP stimulates actin filament elongation by promoting the transfer of profilin-bound actin monomers onto the barbed end of growing actin filaments. Plays a role in actin-based mobility of <i>Listeria monocytogenes</i> in host cells. Regulates actin dynamics in platelets and plays an important role in regulating platelet aggregation.
<b>Tissue specificity</b>	Highly expressed in platelets.
<b>Sequence similarities</b>	Belongs to the Ena/VASP family. Contains 1 WH1 domain.
<b>Domain</b>	The EVH2 domain is comprised of 3 regions. Block A is a thymosin-like domain required for G-actin binding. The KLKR motif within this block is essential for the G-actin binding and for actin polymerization. Block B is required for F-actin binding and subcellular location, and Block C for tetramerization. The WH1 domain mediates interaction with XIRP1.
<b>Post-translational modifications</b>	Major substrate for cAMP-dependent (PKA) and cGMP-dependent protein kinase (PKG) in platelets. The preferred site for PKA is Ser-157, the preferred site for PKG, Ser-239. In ADP-activated platelets, phosphorylation by PKA or PKG on Ser-157 leads to fibrinogen receptor inhibition. Phosphorylation on Thr-278 requires prior phosphorylation on Ser-157 and Ser-239. In response to phorbol ester (PMA) stimulation, phosphorylated by PKC/PRKCA. In response to

thrombin, phosphorylated by both PKC and ROCK1. Phosphorylation at Thr-278 by AMPK does not require prior phosphorylation at Ser-157 or Ser-239. Phosphorylation modulates F-actin binding, actin filament elongation and platelet activation. Carbon monoxide (CO) promotes phosphorylation at Ser-157, while nitric oxide (NO) promotes phosphorylation at Ser-157, but also at Ser-239. Response to NO and CO is blunted in platelets from diabetic patients, and VASP is not phosphorylated efficiently at Ser-157 and Ser-239.

### Cellular localization

Cytoplasm. Cytoplasm > cytoskeleton. Cell junction > focal adhesion. Cell projection > lamellipodium membrane. Cell projection > filopodium membrane. Targeted to stress fibers and focal adhesions through interaction with a number of proteins including MRL family members. Localizes to the plasma membrane in protruding lamellipodia and filopodial tips. Stimulation by thrombin or PMA, also translocates VASP to focal adhesions. Localized along the sides of actin filaments throughout the peripheral cytoplasm under basal conditions.

### Applications

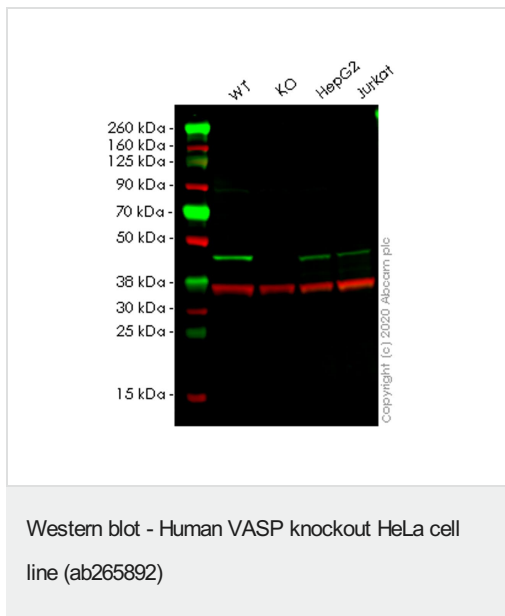
#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab265892 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		Use at an assay dependent concentration. Predicted molecular weight: 39 kDa.

### Images



**All lanes :** Anti-VASP antibody [EPR1337(2)] ([ab109321](#)) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 2 :** VASP knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 3 :** HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate

**Lane 4 :** Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

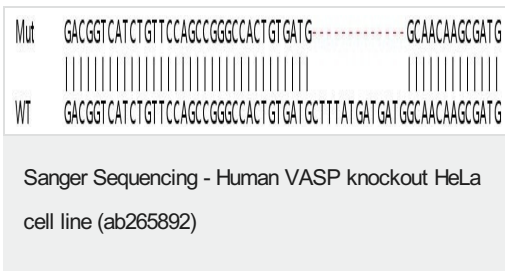
**All lanes :** Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

**Predicted band size:** 39 kDa

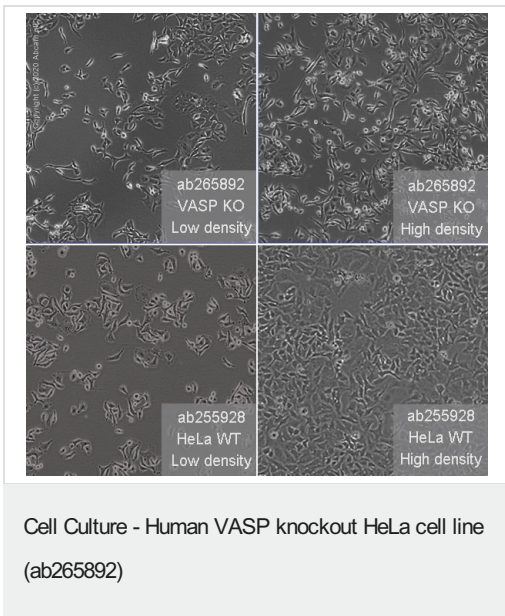
**Observed band size:** 46 kDa

**Lanes 1-4:** Merged signal (red and green). Green - **ab109321** observed at 46 kDa. Red - loading control **ab8245** observed at 36 kDa.

**ab109321** Anti-VASP antibody [EPR1337(2)] was shown to specifically react with VASP in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265892 (knockout cell lysate **ab257792**) was used. Wild-type and VASP knockout samples were subjected to SDS-PAGE. **ab109321** 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.



Homozygous: 13 bp deletion in exon 2.



Representative images of VASP knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS M5000 microscope.

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

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