

Human ENG (CD105) knockout HeLa cell line ab265178

5 Images

Overview

Product name	Human ENG (CD105) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 11 bp deletion in exon 2 and 19 bp deletion in exon 2 and 1 bp insertion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HeLa cell line (ab255928). 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>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>Initial handling guidelines: 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"> 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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 2×10^4 cells/cm². Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if</p>

required.

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

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

Target

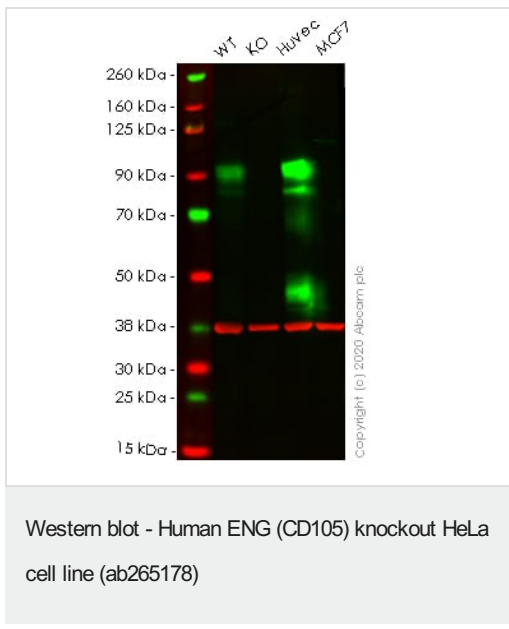
Function	Major glycoprotein of vascular endothelium. May play a critical role in the binding of endothelial cells to integrins and/or other RGD receptors.
Tissue specificity	Endoglin is restricted to endothelial cells in all tissues except bone marrow.
Involvement in disease	Defects in ENG are the cause of hereditary hemorrhagic telangiectasia type 1 (HHT1) [MIM:187300, 108010]; also known as Osler-Rendu-Weber syndrome 1 (ORW1). HHT1 is an autosomal dominant multisystemic vascular dysplasia, characterized by recurrent epistaxis, muco-cutaneous telangiectases, gastro-intestinal hemorrhage, and pulmonary (PAVM), cerebral (CAVM) and hepatic arteriovenous malformations; all secondary manifestations of the underlying vascular dysplasia. Although the first symptom of HHT1 in children is generally nose bleed, there is an important clinical heterogeneity.
Cellular localization	Membrane.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab265178 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: 70 kDa.

Images



All lanes : Anti-CD105 antibody [EPR10145-10] ([ab170943](#)) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : ENG knockout HeLa cell lysate

Lane 3 : HUVEC cell lysate

Lane 4 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

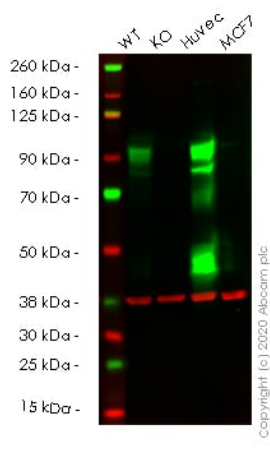
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 70 kDa

Observed band size: 70-120 kDa

ab170943 Anti-CD105 antibody [EPR10145-10] was shown to specifically react with CD105 in HeLa wild type cells. Loss of signal was observed when knockout cell line ab265178 (knockout cell lysate [ab256906](#)) was used. Wild type and CD105 knockout samples were subjected to SDS-PAGE. **ab170943** 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.



Western blot - Human ENG (CD105) knockout HeLa cell line (ab265178)

All lanes : Anti-CD105 antibody [EPR10145-12] ([ab169545](#)) at 1/1000 dilution

- Lane 1** : Wild-type HeLa cell lysate
- Lane 2** : ENG knockout HeLa cell lysate
- Lane 3** : HUVEC cell lysate
- Lane 4** : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 70 kDa

Observed band size: 70-120 kDa

ab169545 Anti-CD105 antibody [EPR10145-12] was shown to specifically react with CD105 in HeLa wild type cells. Loss of signal was observed when knockout cell line ab265178 (knockout cell lysate [ab256906](#)) was used. Wild type and CD105 knockout samples were subjected to SDS-PAGE. **ab169545** 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.



Sanger Sequencing - Human ENG knockout HeLa cell line (ab265178)

Allele-1: 19 bp deletion in exon 2.

Mut	CCAACAGGCTTGCAGAAACAGTCCATTGT-----TGTGGGCCCCGAGAGGGGG
WT	CCAACAGGCTTGCAGAAACAGTCCATTGTGACCTTCAGCCTGTGGGCCCCGAGAGGGGG

Allele-2: 11 bp deletion in exon 2.

Sanger Sequencing - Human ENG knockout HeLa
cell line (ab265178)

Mut	CCAACAGGCTTGCAGAAACAGTCCATTGTGACCTTCAGCCTGTGGGCCCCGAGAGGGGG
WT	CCAACAGGCTTGCAGAAACAGTCCATTGTGACCTTCAGCCTGTGGGCCCCGAGAGGGGG

Allele-3: 1 bp insertion in exon 2.

Sanger Sequencing - Human ENG knockout HeLa
cell line (ab265178)

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