# abcam

# Product datasheet

# Human ENG (CD105) knockout HeLa cell line ab265178

# 画像数5

#### 製品の概要

製品名 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)

アプリケーション **適用あり**: WB

Biosafety level 2

特記事項 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.

**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.

Culture medium: DMEM (High Glucose) + 10% FBS

**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.

- 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 2x10<sup>4</sup> cells/cm<sup>2</sup>. 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% CO<sub>2</sub>. Cultures should be monitored daily.

#### Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of  $2x10^4$  cells/cm<sup>2</sup> is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

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required.

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

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licenses and patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

We will provide viable cells that proliferate on revival.

#### 製品の特性

Number of cells 1 x 10<sup>6</sup> 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

保存方法 Shipped on Dry Ice. Store in liquid nitrogen.

パップァー Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

#### ターゲット情報

機能 Major glycoprotein of vascular endothelium. May play a critical role in the binding of endothelial

cells to integrins and/or other RGD receptors.

組織特異性 Endoglin is restricted to endothelial cells in all tissues except bone marrow.

**関連疾患** 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.

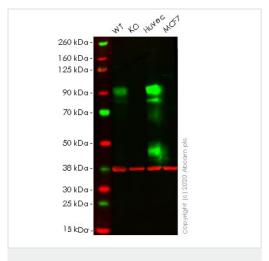
細胞内局在 Membrane.

## アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab265178の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 70 kDa.

#### 画像



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

**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**

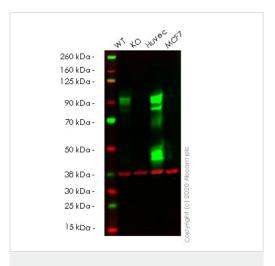
**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

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 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.



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

**All lanes :** Anti-CD105 antibody [EPR10145-12] (<u>ab169545</u>) 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 lgG 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 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.



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

Allele-1: 19 bp deletion in exon 2.

Mut	CCAACAGGTCTTGCAGAAACAGTCCATTGTTGTGGGCCCCGAGAGGGGC			
WT	CCAACAGGTCTTGCAGAAACAGTCCATTGTGACCTTCAGCCTGTGGGCCCCGAGAGGGGGC			
Sanger Sequencing - Human ENG knockout HeLa				
Sanger Sequencing - Human ENG Knockout neta				
cell line (ab265178)				

Allele-2: 11 bp deletion in exon 2.

Mut	CCAACAGGTCTTGCAGAAACAGTCCATTGTT	GACCTTCAGCCTGTGGGCCCCGAGAGGGG		
VA/T				
WT	CCAACAGGTCTTGCAGAAACAGTCCATTGT	UACCI I CAUCCI UI UUUCCCCUAUAUUUU		
Sanger Sequencing - Human ENG knockout HeLa				

cell line (ab265178)

Allele-3: 1 bp insertion in exon 2.

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