abcam

Product datasheet

Human VIM (Vimentin) knockout HeLa cell line ab255446

3 图像

概述

产品名称	人VIM (Vimentin) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** WB
Biosafety level	2
常规说明	**Recommended control:** Human wild-type HeLa cell line (**ab255448**). 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 $2x10^4$ cells/cm^2. 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_2. 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^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

性能

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
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2.
组织特异性	Highly expressed in fibroblasts, some expression in T- and B-lymphocytes, and little or no expression in Burkitt's lymphoma cell lines. Expressed in many hormone-independent mammary carcinoma cell lines.
疾病相关	Cataract 30
序列相似性	Belongs to the intermediate filament family.
结构域	The central alpha-helical coiled-coil rod region mediates elementary homodimerization. The [IL]-x-C-x-x-[DE] motif is a proposed target motif for cysteine S-nitrosylation mediated by the iNOS-S100A8/A9 transnitrosylase complex.
翻译后修饰	Filament disassembly during mitosis is promoted by phosphorylation at Ser-55 as well as by nestin (By similarity). One of the most prominent phosphoproteins in various cells of mesenchymal origin. Phosphorylation is enhanced during cell division, at which time vimentin filaments are significantly reorganized. Phosphorylation by PKN1 inhibits the formation of filaments. Phosphorylated at Ser-56 by CDK5 during neutrophil secretion in the cytoplasm. Phosphorylated by STK33. O-glycosylated during cytokinesis at sites identical or close to phosphorylation sites, this interferes with the phosphorylation status. S-nitrosylation is induced by interferon-gamma and oxidatively-modified low-densitity lipoprotein

	(LDL(ox)) possibly implicating the iNOS-S100A8/9 transnitrosylase complex.
细胞定位	Cytoplasm.
形式	Vimentin is found in connective tissue and in the cytoskeleton.

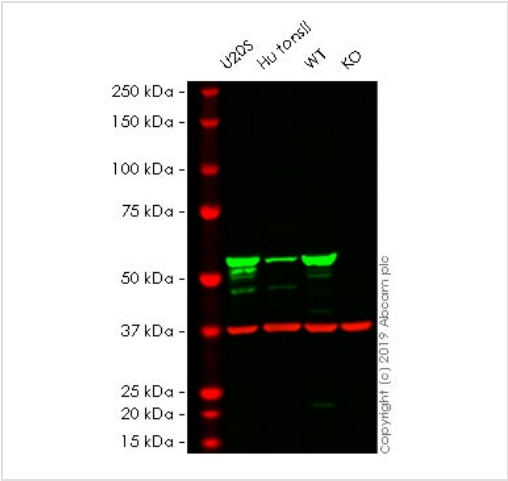
应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab255446**于以下的**经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 53 kDa.

图片



Western blot - Human VIM (Vimentin) knockout HeLa cell line (ab255446)

All lanes : Anti-Vimentin antibody [V9] - Cytoskeleton Marker (**ab8069**) at 1 µg/ml

Lane 1 : U-2 OS (Human bone osteosarcoma epithelial cell line) cell lysate
Lane 2 : Human tonsil cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : VIM knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) at 1/20000 dilution

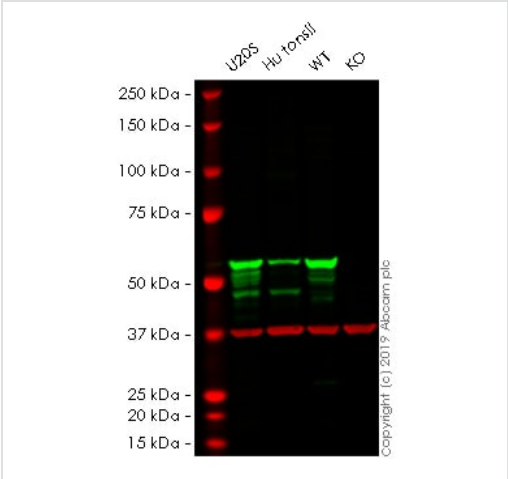
Performed under reducing conditions.

Predicted band size: 53 kDa
Additional bands at: 50 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab8069** observed at 53 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab8069 was shown to react with Vimentin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255446

(knockout cell lysate **ab263775**) was used. Wild-type and Vimentin knockout samples were subjected to SDS-PAGE. **ab8069** and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 µg/ml and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human VIM (Vimentin) knockout HeLa cell line (ab255446)

All lanes : Anti-Vimentin antibody [SP20] (**ab16700**) at 1/100 dilution

Lane 1 : U20S cell lysate
Lane 2 : Human tonsil cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : VIM knockout HeLa 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: 53 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab16700** observed at 53 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab16700 was shown to react with Vimentin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255446 (knockout cell lysate **ab263775**) was used. Wild-type and Vimentin knockout samples were subjected to SDS-PAGE. **ab16700** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 100 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 VIM knockout HeLa cell line (ab255446)

Homozygous: Insertion of the selection cassette in exon 2.

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