

Human MKI67 (Ki67) knockout HeLa cell line ab255407

2 Images

Overview

Product name	Human MKI67 (Ki67) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 7 bp deletion in exon 7
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 (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.</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.</p> <p>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

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 WWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
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	Required to maintain individual mitotic chromosomes dispersed in the cytoplasm following nuclear envelope disassembly (PubMed:27362226). Associates with the surface of the mitotic chromosome, the perichromosomal layer, and covers a substantial fraction of the chromosome surface (PubMed:27362226). Prevents chromosomes from collapsing into a single chromatin mass by forming a steric and electrostatic charge barrier: the protein has a high net electrical charge and acts as a surfactant, dispersing chromosomes and enabling independent chromosome motility (PubMed:27362226). Binds DNA, with a preference for supercoiled DNA and AT-rich DNA (PubMed:10878551). Does not contribute to the internal structure of mitotic chromosomes (By similarity). May play a role in chromatin organization (PubMed:24867636). It is however unclear whether it plays a direct role in chromatin organization or whether it is an indirect consequence of its function in maintaining mitotic chromosomes dispersed.
Sequence similarities	Contains 1 FHA domain. Contains 16 K167R repeats. Contains 1 PP1-binding domain.
Developmental stage	Expression occurs preferentially during late G1, S, G2 and M phases of the cell cycle, while in cells in G0 phase the antigen cannot be detected (at protein level) (PubMed:6206131). Present at highest level in G2 phase and during mitosis (at protein level). In interphase, forms fiber-like structures in fibrillarin-deficient regions surrounding nucleoli (PubMed:2674163, PubMed:8799815).
Post-translational modifications	Phosphorylated. Hyperphosphorylated in mitosis (PubMed:10502411, PubMed:10653604). Hyperphosphorylated form does not bind DNA.
Cellular localization	Chromosome. Nucleus. Nucleus, nucleolus. Associates with the surface of the mitotic chromosome, the perichromosomal layer, and covers a substantial fraction of the mitotic

chromosome surface (PubMed:27362226). Associates with satellite DNA in G1 phase (PubMed:9510506). Binds tightly to chromatin in interphase, chromatin-binding decreases in mitosis when it associates with the surface of the condensed chromosomes (PubMed:15896774, PubMed:22002106). Predominantly localized in the G1 phase in the perinucleolar region, in the later phases it is also detected throughout the nuclear interior, being predominantly localized in the nuclear matrix (PubMed:22002106).

Applications

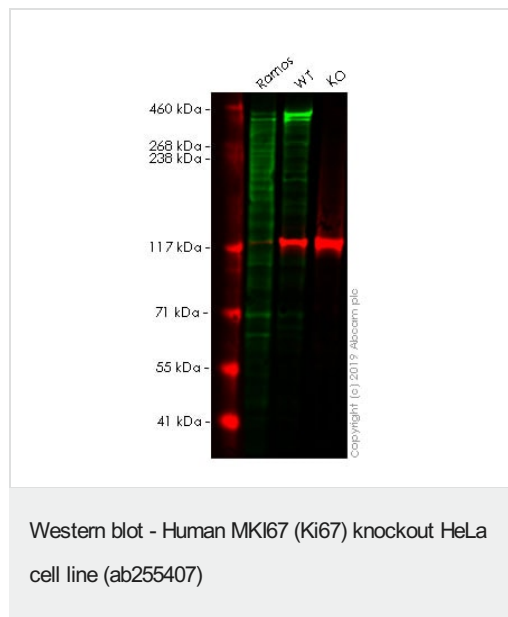
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab255407 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: 358 kDa.

Images



All lanes : Anti-Ki67 antibody [SP6] (**ab16667**) at 1/100 dilution

Lane 1 : Ramos cell lysate

Lane 2 : Wild-type HeLa cell lysate

Lane 3 : MKI67 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.

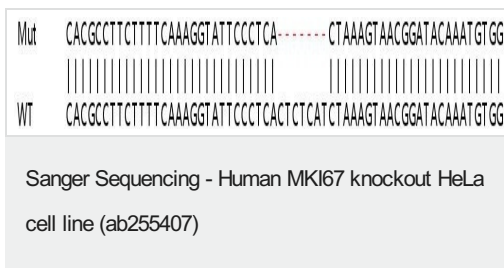
Predicted band size: 358 kDa

Additional bands at: 124 kDa (possible Loading Control)

Lanes 1 - 3: Merged signal (red and green). Green - **ab16667** observed at 359 kDa. Red - loading control, **ab130007** observed at 125 kDa.

ab16667 was shown to react with Ki67 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255407 (knockout cell lysate **ab263762**) was used. Wild-type and Ki67

knockout samples were subjected to SDS-PAGE. **ab16667** and Anti-Vinculin antibody [VIN-54] (**ab130007**) 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.



Homozygous: 7 bp deletion in exon 7.

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