abcam

Product datasheet

Human MAPK7 (ERK5) knockout HeLa cell line ab265508

2 Images

Overview

Product name	Human MAPK7 (ERK5) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**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 $2x10^4$ 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.
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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
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	Plays a role in various cellular processes such as proliferation, differentiation and cell survival. The upstream activator of MAPK7 is the MAPK kinase MAP2K5. Upon activation, it translocates to the nucleus and phosphorylates various downstream targets including MEF2C. EGF activates MAPK7 through a Ras-independent and MAP2K5-dependent pathway. May have a role in muscle cell differentiation. May be important for endothelial function and maintenance of blood vessel integrity. MAP2K5 and MAPK7 interact specifically with one another and not with MEK1/ERK1 or MEK2/ERK2 pathways.
Tissue specificity	Expressed in many adult tissues. Abundant in heart, placenta, lung, kidney and skeletal muscle. Not detectable in liver.
Sequence similarities	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. Contains 1 protein kinase domain.
Domain	The second proline-rich region may interact with actin targeting the kinase to a specific location in the cell. The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.
Post-translational modifications	Dually phosphorylated on Thr-219 and Tyr-221, which activates the enzyme (By similarity). Autophosphorylated in vitro on threonine and tyrosine residues when the C-terminal part of the kinase, which could have a regulatory role, is absent.
Cellular localization	Cytoplasm. Nucleus. Translocates to the nucleus upon activation.

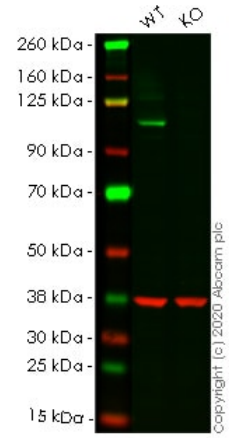
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265508 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: 88 kDa.

Images



Western blot - Human MAPK7 (ERK5) knockout HeLa cell line (ab265508)

All lanes : Anti-ERK5 antibody [EP791Y] (**ab40809**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MAPK7 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 115 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab40809** observed at 115 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab40809 was shown to react with ERK5 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265508 (knockout cell lysate **ab258042**) was used. Wild-type HeLa and MAPK7 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab40809** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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 MAPK7 knockout HeLa cell line (ab265508)

Homozygous: 1 bp insertion in exon 4.

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