

Product datasheet

Human MAPK1 (ERK2) knockout HeLa cell line ab265052

[5 Images](#)

Overview

Product name	Human MAPK1 (ERK2) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 2 and Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: ICC/IF, 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.</p>

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 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 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	<p>Involved in both the initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors such as ELK1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2 (MAP2). Phosphorylates SPZ1 (By similarity). Phosphorylates heat shock factor protein 4 (HSF4) and ARHGEF2.</p> <p>Acts as a transcriptional repressor. Binds to a [GC]AAA[GC] consensus sequence. Repress the expression of interferon gamma-induced genes. Seems to bind to the promoter of CCL5, DMP1, IFIH1, IFITM1, IRF7, IRF9, LAMP3, OAS1, OAS2, OAS3 and STAT1. Transcriptional activity is independent of kinase activity.</p>
Sequence similarities	<p>Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily.</p> <p>Contains 1 protein kinase domain.</p>
Domain	The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.
Post-translational modifications	Dually phosphorylated on Thr-185 and Tyr-187, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-187.
Cellular localization	Nucleus.

Applications

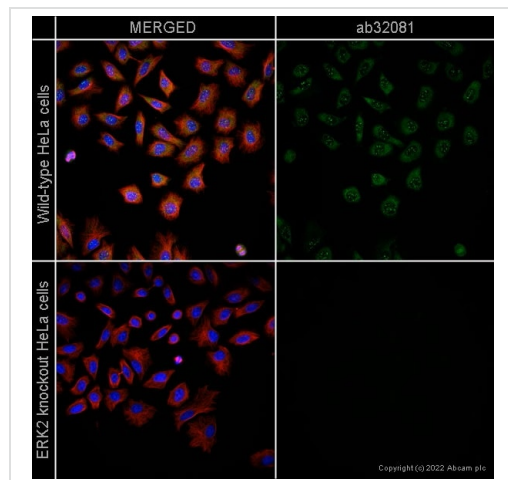
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab265052 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

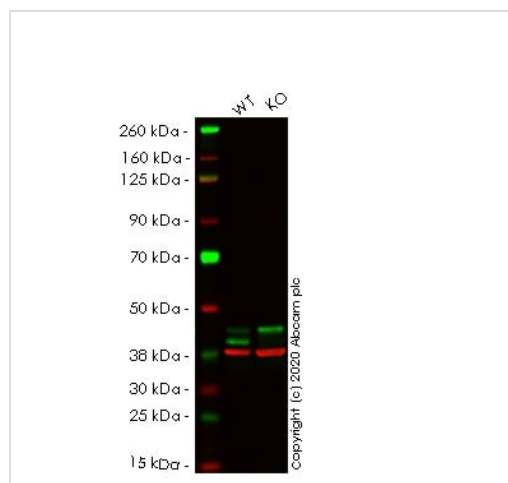
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 41 kDa.

Images



Immunocytochemistry/ Immunofluorescence -
Human MAPK1 (ERK2) knockout HeLa cell line
(ab265052)

ab32081 staining ERK2 in wild-type HeLa cells (top panel) and ERK2 knockout HeLa cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with **ab32081** at 0.2µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Human MAPK1 knockout HeLa cell
line (ab265052)

All lanes : Anti-ERK1 + ERK2 antibody [EPR17526] (**ab184699**)
at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : MAPK1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

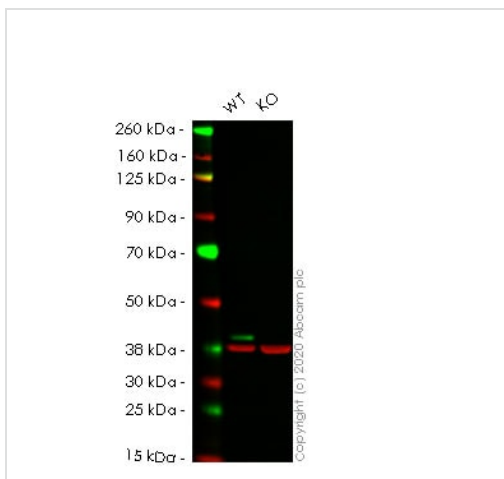
Performed under reducing conditions.

Predicted band size: 41 kDa

Observed band size: 44 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab184699** observed at 44 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab184699 Anti-ERK1 + ERK2 antibody [EPR17526] was shown to specifically react with ERK2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265052 (knockout cell lysate **ab257525**) was used. Wild-type and ERK2 knockout samples were subjected to SDS-PAGE. **ab184699** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 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 MAPK1 knockout HeLa cell line (ab265052)

All lanes : Anti-ERK2 antibody [E460] (**ab32081**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : MAPK1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 41 kDa

Observed band size: 41 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab32081** observed at 41 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab32081 Anti-ERK2 antibody [E460] was shown to specifically react with ERK2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265052 (knockout cell lysate **ab257525**) was used. Wild-type and ERK2 knockout samples were subjected to SDS-PAGE. **ab32081** 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.

```
Mut  AAGCGCAGTAAGATTTTATCTCCCTCAGG- TTCTCTGGCAGTAGGTCTGGTCTCAAAG
      |||
WT   AAGCGCAGTAAGATTTTATCTCCCTCAGGTTCTCTGGCAGTAGGTCTGGTCTCAAAG
```

Sanger Sequencing - Human MAPK1 knockout
HeLa cell line (ab265052)

Allele-1: 1 bp deletion in exon 2.

```
Mut  AGATTTTATCTCCCTCAGG*****Insertion***** GTTCTCTGGCAGTAGGTCTG
      |||
WT   AGATTTTATCTCCCTCAGG                GTTCTCTGGCAGTAGGTCTG
```

Sanger Sequencing - Human MAPK1 knockout
HeLa cell line (ab265052)

Allele-2: Insertion of the selection cassette in exon 2.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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