abcam

Product datasheet

Human MAPK1 (ERK2) knockout HeLa cell line ab265052

画像数 5

製品の概要

製品名	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)
アプリケーション	**適用あり:** ICC/IF, WB
Biosafety level	2
特記事項	**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.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	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. 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.
配列類似性	Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. MAP kinase subfamily. Contains 1 protein kinase domain.
ドメイン	The TXY motif contains the threonine and tyrosine residues whose phosphorylation activates the MAP kinases.
翻訳後修飾	Dually phosphorylated on Thr-185 and Tyr-187, which activates the enzyme. Dephosphorylated by PTPRJ at Tyr-187.
細胞内局在	Nucleus.

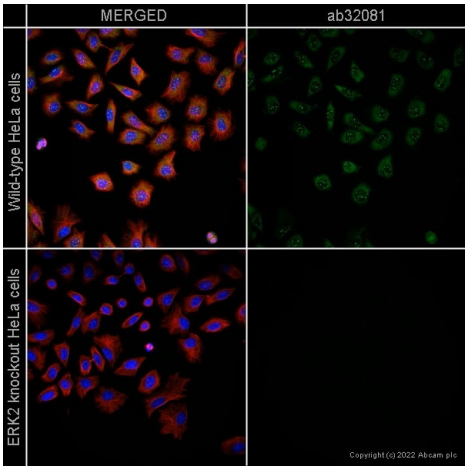
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab265052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。

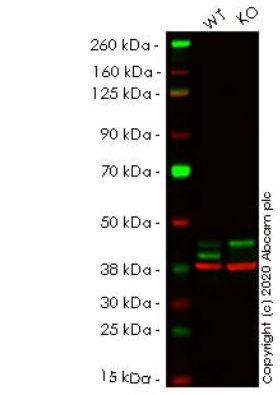
アプリケーション	Abreviews	特記事項
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 41 kDa.

画像



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.

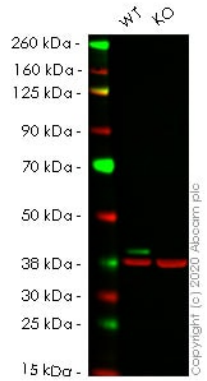
Performed under reducing conditions.

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.

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

Allele-1: 1 bp deletion in exon 2.

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

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

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