abcam

Product datasheet

Human RBPMS knockout HeLa cell line ab264697

4 Images

Overview

Product name	Human RBPMS 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 44 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing
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.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

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	Acts as a coactivator of transcriptional activity. Required to increase TGFB1/Smad-mediated transactivation. Acts through SMAD2, SMAD3 and SMAD4 to increase transcriptional activity. Increases phosphorylation of SMAD2 and SMAD3 on their C-terminal SSXS motif, possibly through recruitment of TGFBR1. Promotes the nuclear accumulation of SMAD2, SMAD3 and SMAD4 proteins. Binds to poly(A) RNA.
Tissue specificity	Ubiquitously expressed, at various levels depending on the isoform and the tissue.
Sequence similarities	Contains 1 RRM (RNA recognition motif) domain.
Domain	The RRM domain is necessary for interaction with SMAD4. Both the RRM domain and the C-terminus are required for TGFB1/Smad-mediated transactivation activity.
Cellular localization	Nucleus. Cytoplasm.

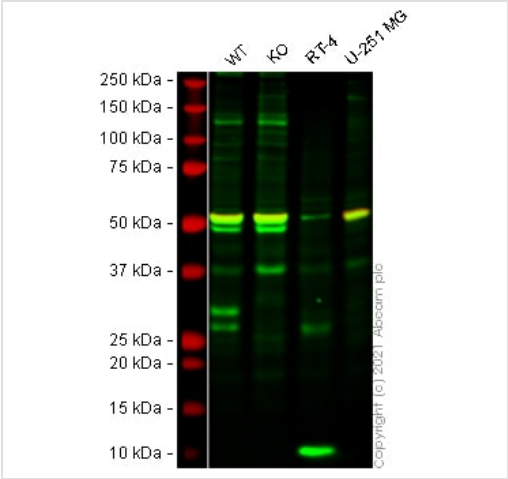
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab264697 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.

Images



Western blot - Human RBPMS knockout HeLa cell line (ab264697)

All lanes : Anti-RBPMS antibody (**ab194213**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RBPMS Knockout HeLa cell lysate
Lane 3 : RT-4 cell lysate
Lane 4 : U-251 MG cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

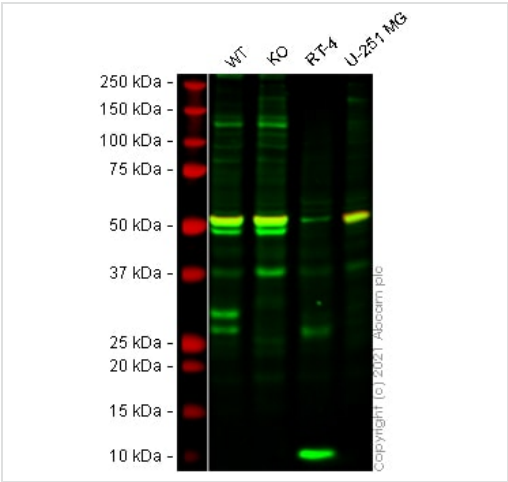
Observed band size: 30 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab194213** observed at 30 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab194213 was shown to react with RBPMS in wild-type HeLa cells in western blot. Here no band was observed in the RBPMS knockout cell line ab264697 (RBPMS knockout cell lysate **ab258631**), but other antibodies have shown bands in this lysate below 30 kDa that may represent truncated forms and cleaved fragments. This has not been investigated further. HeLa wild-type and RBPMS knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab194213** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Sanger Sequencing - Human RBPMS knockout HeLa cell line (ab264697)

Allele-1: 1 bp deletion in exon 2.



Western blot - Human RBPMS knockout HeLa cell line (ab264697)

All lanes : Anti-RBPMS antibody (**ab152101**) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RBPMS Knockout HeLa cell lysate
Lane 3 : RT-4 cell lysate
Lane 4 : U-251 MG cell lysate

Lysates/proteins at 20 µg per lane.

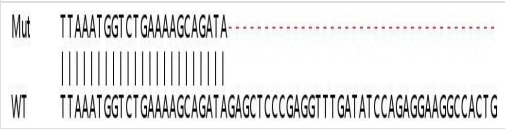
Performed under reducing conditions.

Observed band size: 30 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab152101** observed at 30 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

Lanes 1 - 4: Merged signal (red and green). Green - **ab152101** observed at 30 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab152101 was shown to react with RBPMS in wild-type HeLa cells in western blot. The bands observed in RBPMS knockout cell line ab264697 (RBPMS knockout cell lysate **ab258631**) below 30 kDa may represent truncated forms and cleaved fragments. This has not been investigated further and the functional properties of the gene product have not been determined. HeLa wild-type and RBPMS knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab152101** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Sanger Sequencing - Human RBPMS knockout HeLa cell line (ab264697)

Allele-2: 44 bp deletion in exon 2.

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