abcam

Product datasheet

Human MET (Met (c-Met)) knockout HeLa cell line ab265961

4 Images

Overview

Product name	Human MET (Met (c-Met)) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 5 bp deletion in exon 2
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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Properties

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
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Receptor for hepatocyte growth factor and scatter factor. Has a tyrosine-protein kinase activity. Functions in cell proliferation, scattering, morphogenesis and survival.
Involvement in disease	Note=Activation of MET after rearrangement with the TPR gene produces an oncogenic protein. Note=Defects in MET may be associated with gastric cancer. Defects in MET are a cause of hepatocellular carcinoma (HCC) [MIM:114550]. Defects in MET are a cause of renal cell carcinoma papillary (RCCP) [MIM:605074]. It is a subtype of renal cell carcinoma tending to show a tubulo-papillary architecture formed by numerous, irregular, finger-like projections of connective tissue. Renal cell carcinoma is a heterogeneous group of sporadic or hereditary carcinoma derived from cells of the proximal renal tubular epithelium. It is subclassified into common renal cell carcinoma (clear cell, non-papillary carcinoma), papillary renal cell carcinoma, chromophobe renal cell carcinoma, collecting duct carcinoma with medullary carcinoma of the kidney, and unclassified renal cell carcinoma. Note=A common allele in the promoter region of the MET shows genetic association with susceptibility to autism in some families. Functional assays indicate a decrease in MET promoter activity and altered binding of specific transcription factor complexes. Note=MET activating mutations may be involved in the development of a highly malignant, metastatic syndrome known as cancer of unknown primary origin (CUP) or primary occult malignancy. Systemic neoplastic spread is generally a late event in cancer progression. However, in some instances, distant dissemination arises at a very early stage, so that metastases reach clinical relevance before primary lesions. Sometimes, the primary lesions cannot be identified in spite of the progresses in the diagnosis of malignancies.

Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. Contains 3 IPT/TIG domains. Contains 1 protein kinase domain. Contains 1 Sema domain.
Domain	The kinase domain is involved in SPSB1 binding.
Post-translational modifications	Dephosphorylated by PTPRJ at Tyr-1349 and Tyr-1365.
Cellular localization	Membrane.

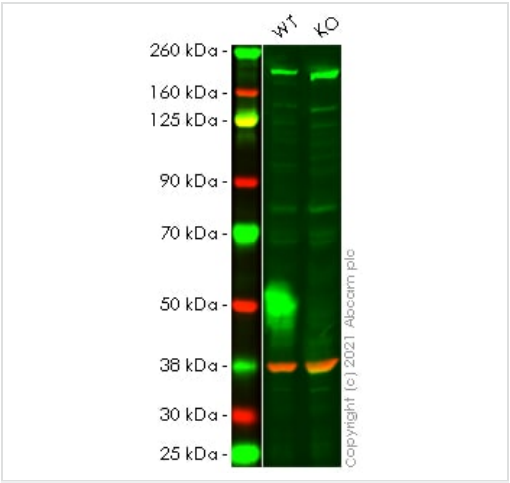
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab265961 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: 155 kDa.

Images



Western blot - Human MET (Met (c-Met)) knockout HeLa cell line (ab265961)

All lanes : Anti-Met (c-Met) antibody [EP1454Y] - N-terminal (ab51067) at 1/1000 dilution

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

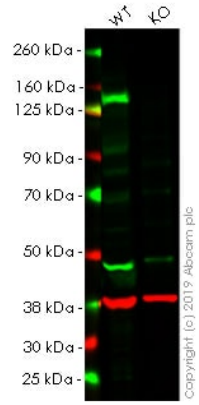
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 155 kDa
Observed band size: 50 kDa

False colour image of Western blot: Anti-Met (c-Met) antibody [EP1454Y] - N-terminal staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab51067 was shown to bind specifically to the alpha chain of c-Met. A band was observed at 50 kDa in wild-type HeLa cell lysates with no signal observed at this size in MET knockout cell line ab265961 (knockout cell lysate ab256991). To generate this image, wild-type

and MET knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Human MET knockout HeLa cell line (ab265961)

All lanes : Anti-Met (c-Met) antibody [EP1454Y] - N-terminal (ab51067) at 1/1000 dilution

Lane 1 : Wild-type HeLa
Lane 2 : MET knockout HeLa

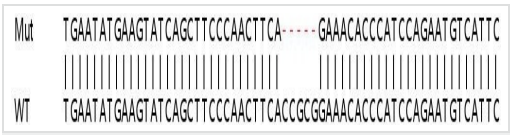
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/20000 dilution

Predicted band size: 155 kDa
Observed band size: 37 kDa

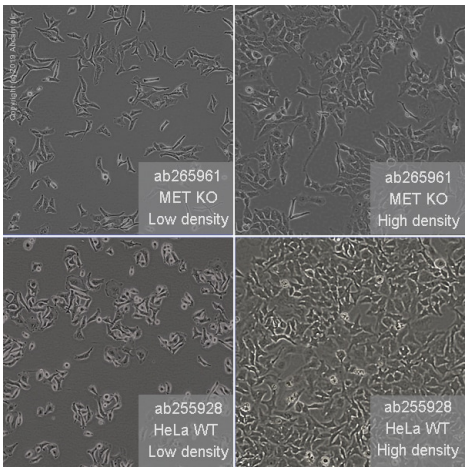
Lanes 1 - 2: Merged signal (red and green). Green - ab216574 observed at 156 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab216574 was shown to react with Met (c-Met) in wild-type HeLa. Loss of signal was observed when knockout cell line ab265961 (knockout cell lysate ab256991) was used. Wild-type and Met (c-Met) knockout samples were subjected to SDS-PAGE. ab216574 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 MET knockout HeLa cell line (ab265961)

Homozygous: 5 bp deletion in exon 2.



Cell Culture - Human MET (Met (c-Met)) knockout HeLa cell line (ab265961)

Representative images of MET knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

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