abcam

Product datasheet

Human MET (c-Met) knockout HeLa cell lysate ab256991

3 Images

Overview

Product name	Human MET (c-Met) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 5 bp deletion in exon2.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found here. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. See here for more information on knockout cell lysates. Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. 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.
Tested applications	**Suitable for:** WB

Properties

Storage instructions	Store at -80°C. Please refer to protocols.

Components	1 kit
ab262008 - Human MET knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

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

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.

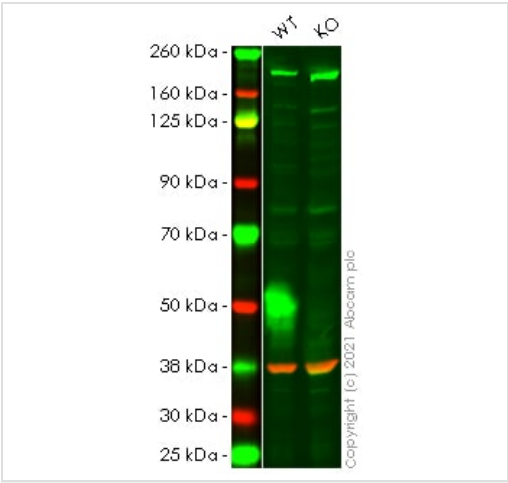
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab256991 in the following tested applications.

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

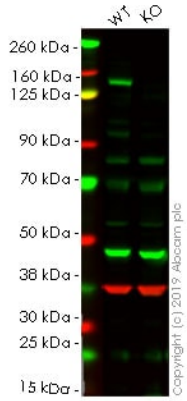
Application	Abreviews	Notes
WB		Use at an assay dependent concentration.

Images



Western blot - Human MET (c-Met) knockout HeLa cell lysate (ab256991)

Lane 1: Wild-type HeLa cell lysate 20 µg
Lane 2: MET knockout HeLa cell lysate 20 µg
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.



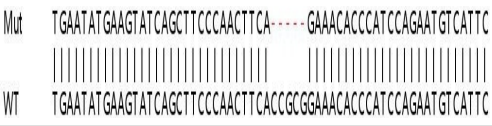
Western blot - Human MET knockout HeLa cell lysate (ab256991)

Lane 1: Wild-type HeLa cell lysate (20 µg)

Lane 2: MET knockout HeLa cell lysate (20 µg)

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 lysate (ab256991)

Homozygous: 5 bp deletion in exon2

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