abcam

Product datasheet

Human MRAS knockout HeLa cell lysate ab257541

3 Images

Overview	
Product name	Human MRAS 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, 1 bp insertion in exon3 and 2 bp deletion in exon3.
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). <i>This means that the protein of interest is denatured.</i> If you require a native form of the protein please use the live cell version - found <u>here</u> . 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.
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Tested applications	Suitable for: WB

Properties

Storage instructions	Store at -80°C. Please refer to protocols.	
Components		1 kit
ab261008 - Human MRAS 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	May serve as an important signal transducer for a novel upstream stimuli in controlling cell proliferation. Weakly activates the MAP kinase pathway.
Tissue specificity	Expression highly restricted to the brain and heart.
Sequence similarities	Belongs to the small GTPase superfamily. Ras family.
Cellular localization	Cell membrane.

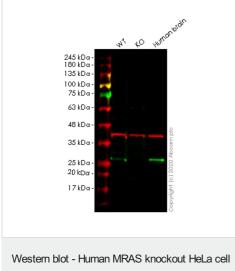
Applications

The Abpromise guaranteeOur Abpromise guaranteecovers the use of ab257541 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: 24 kDa.

Images



lysate (ab257541)

Mut WT	AGCCATCCCCCGTGCGCATGTATTGCTCGCATGGCGCTGAATTCCTCCTGCCCAGCTG
Sa	nger Sequencing - Human MRAS knockout HeLa

AGCCAT CCCCCGT GCGCAT GTATT GCT CGCCGCAT GGCGCT GAATT CCT CCT GCCCAGCT

AGCCATCCCCCGTGCGCATGTATTGCTC CCGCATGGCGCTGAATTCCTCCTGCCCAGCT

Sanger Sequencing - Human MRAS knockout HeLa

cell lysate (ab257541)

cell lysate (ab257541)

Mut

WT

Lane 1: Wild-type HeLa cell lysate (20 ug) Lane 2: MRAS knockout HeLa cell lysate (20 ug) Lane 3: Human brain tissue lysate (20 ug)

<u>ab176570</u> was shown to specifically react with MRas in wild-type HeLa cells. Loss of signal was observed when knockout cell line <u>ab265510</u> (knockout cell lysate ab257541) was used. Wild-type and MRas knockout samples were subjected to SDS-PAGE. <u>ab176570</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Allele-1: 2 bp deletion in exon3

Allele-2: 1 bp insertion in exon3

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