abcam

Product datasheet

Human FLOT1 (Flotillin 1) knockout HEK-293T cell lysate ab257109

3 Images

Overview

Product name Human FLOT1 (Flotillin 1) knockout HEK-293T cell lysate

Product overview

Knockout cell lysate achieved by CRISPR/Cas9.

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 8 bp deletion in exon10.

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Reconstitution notesTo 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.

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relevant patents please refer to our <u>limited use license</u> and <u>patent pages</u>.

Tested applications Suitable for: WB

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Properties

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

Components	1 kit
ab262061 - Human FLOT1 knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01:

7, 9.3 TPOX: 11 CSF1PO: 11, 12

Target

Function May act as a scaffolding protein within caveolar membranes, functionally participating in formation

of caveolae or caveolae-like vesicles.

Sequence similaritiesBelongs to the band 7/mec-2 family. Flotillin subfamily.

Cellular localization Cell membrane. Membrane > caveola. Melanosome. Endosome. Membrane-associated protein

of caveolae. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

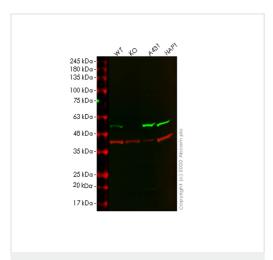
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab257109 in the following tested applications.

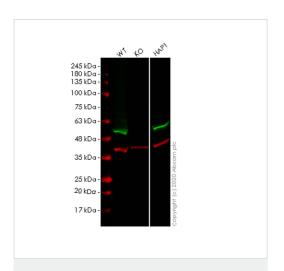
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 47 kDa.

Images



Western blot - Human FLOT1 knockout HEK293T cell lysate (ab257109)



Western blot - Human FLOT1 knockout HEK293T cell lysate (ab257109)

Lane 1: Wild-type HEK293T cell lysate (20 ug)

Lane 2:FLOT1 knockout HEK293T cell lysate (20 ug)

Lane 3:A431 cell lysate (20 ug)

Lane 4: HAP1 cell lysate (20 ug)

<u>ab78178</u> was shown to specifically react with Flotillin 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line <u>ab267276</u> (knockout cell lysate ab257109) was used. Wild-type and Flotillin 1 knockout samples were subjected to SDS-PAGE. <u>ab78178</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) 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 (<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.

Lane 1: Wild-type HEK293T cell lysate (20 ug)

Lane 2:FLOT1 knockout HEK293T cell lysate (20 ug)

Lane 3:HAP1 cell lysate (20 ug)

ab133497 was shown to specifically react with Flotillin 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab267276 (knockout cell lysate ab257109) was used. Wild-type and Flotillin 1 knockout samples were subjected to SDS-PAGE. ab133497 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.

	Mut	TGCCTCTTAACTCACCCGCGGCTTCTGCCTCCGCCTGCATAATTAGTTGGGD				
	WT	TGCCTCTTAACTCACCCGCACAGACGCGGCTTCTGCCTCCGCCTGCATAATTAGTTGGGD				
Sanger Sequencing - Human FLOT1 knockout						
	danger dequarioning - Flamari i Ed i i Knockdat					
	HE	K293T cell lysate (ab257109)				

Homozygous: 8 bp deletion in exon10

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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