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Datasheet for W09-K01-MT9

Human ROBO1 Knockout A549 Cell Lysate

Overview

Description:	Human ROBO1 Knockout A549 Lysate - W09-K01-MT9
Item No.:	W09-K01-MT9
Size:	100 µg
Applications:	SDS-PAGE, WB
Origin:	Human

Product Details

Background:	ROBO-1 (also called Roundabout homolog 1 precursor and Deleted in U twenty twenty (DUTT)) functions as a receptor for SLIT1 and SLIT2. The SLIT proteins are thought to act as a molecular guidance cue in cellular migration, including axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development. In axon growth cones, the silencing of the attractive effect of NTN1 by SLIT2 may require the formation of a ROBO1-DCC complex. ROBO-1 may also be required for lung development. ROBO-1 is a type I membrane protein. ROBO-1 is a widely expressed protein with the exception of the kidney. Defects in ROBO1 may be a cause of breast and lung cancer. ROBO-1 maps within a region of overlapping homozygous deletions characterized in both small cell lung cancer cell lines (SCLC) and in a breast cancer cell line.
Synonyms:	rabbit anti-ROBO1 antibody, ROBO 1, ROBO-1, hROBO-1, Roundabout homolog 1, Deleted in U twenty twenty, DUTT1, DUTT-1
Species of Origin:	Human
Clone ID:	Clone 15

Target Details

Gene Name:	ROBO1

Purity/Specificity:	ROBO1 knockout A549 cells were grown in Dulbecco's medium supplemented with 10% fetal bovine serum. Cells were washed with PBS and then incubated on ice in modified RIPA buffer to lyse the cells. Protein integrity was ensured using a cocktail of protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases (0.1 mM AEBSF HCl, 0.08 µM Aprotinin, 5 µM Bestatin, 1.5 µM E-64, 2 µM Leupeptin Hemisulfate, 1 µM Pepstatin A). Phosphatase inhibitors 1 mM NaF and 1 mM Na3VO4 were also added. Cell debris was removed by centrifugation. Protein concentration was determined by BCA using a commercially available kit. Protein concentration was adjusted to 2 mg/ml with modified 1X RIPA buffer. ROBO1 knockout A549 Clone 15 contains knockout deletions on all three copies of the ROBO1 gene in A549 cells. Each copy contains the same 23bp deletion induced by CRISPR/Cas 9. The deletion occurs in exon 2 and disrupts the sequence encoding amino acids 62 through 69, causing the downstream amino acid sequences to shift out of frame resulting in early stop codons. Validated by Sanger sequencing and Western blot.
Relevant Links:	• UniProtKB - Q9Y6N7

Application Details

Tested Applications:	SDS-PAGE, WB
Application Note:	Human ROBO1 Knockout A549 Cell Lysate has been tested by SDS-PAGE and western blot and is suitable for use in Western blot, ELISA, Immunoprecipitation and ChIP. No detection of expected band at ~181kDa is observed in ROBO1 knockout A549 when compared with unmodified A549 cell lysates by Western blot.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ChIP:	User Optimized
ELISA:	User Optimized
IP:	User Optimized
WB:	User Optimized

Cell Line Data

Cell Line:	Human A549 (lung epithelial carcinoma)
Lysate Fractionation:	Whole Cell Lysate
Lysate Stimulation:	Not Stimulated
Culture Type:	Tissue Culture
Induction:	None (Control)

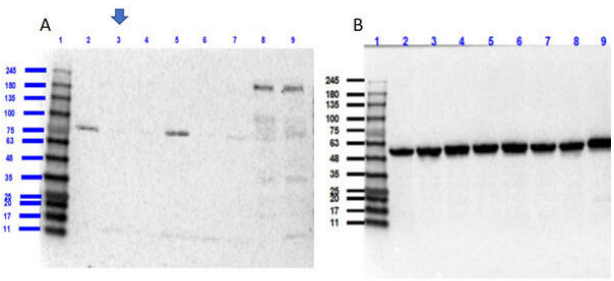
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	2.0 mg/mL by BCA assay
Buffer:	1X RIPA Buffer with HALT Protease and Phosphatase Inhibitors
Preservative:	None
Stabilizer:	None

Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -70° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Western Blot
Western Blot of Human ROBO Knockout A549 Cell Lysate. Lane 1: Opal Prestained MW Marker (p/n MB-210-0500). Lane 2: A549 ROBO KO Clone 5. Lane 3: A549 ROBO KO Clone 15. Lane 4: A549 ROBO KO Clone 17. Lane 5: A549 ROBO KO Clone 18. Lane 6: A549 ROBO KO Clone 21. Lane 7: A549 ROBO1 KO Bulk. Lane 8: A549 WCL Parental (p/n W09-001-372). Lane 9: MCF-7 WCL (p/n W09-000-360). Load: 35µg lysate/lane. Primary Antibody [Blot A] Anti-ROBO1 (p/n 600-401-692) ~181kDa; [Blot B] stripped and re-probed with Anti-Tubulin (p/n 200-301-880) ~50kDa; at 1µg/mL overnight at 2-8°C. Secondary Antibody: Goat Anti-Rabbit IgG HRP at 1:70,000 for 30min at RT. Block: TTBS/Casein at RT. No detection of expected band at ~181kDa is observed in ROBO1 knockout A549 when compared with unmodified A549 cell lysates by Western blot.

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