Product Datasheet

ROBO1 Knockout A549 Cell Lysate
NBP3-18631

Unit Size: 100 ug

Store at -70C. Avoid freeze-thaw cycles.

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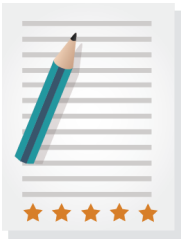


NBP3-18631

ROBO1 Knockout A549 Cell Lysate

Product Information	
Unit Size	100 ug
Concentration	mg/ml
Storage	Store at -70C. Avoid freeze-thaw cycles.
Preservative	No Preservative
Purity	Multi-step
Buffer	1X RIPA Buffer with HALT Protease and Phosphatase Inhibitors

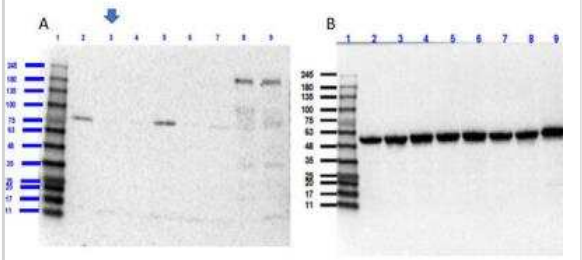
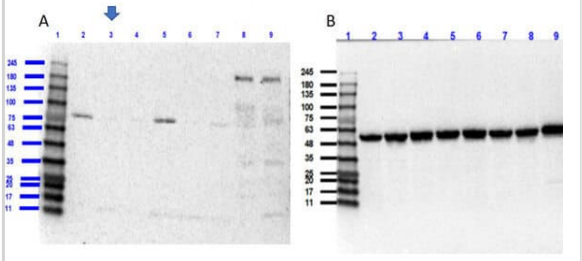
Product Description	
Description	ROBO1 knockout A549 cells were grown in Dulbecco's medium supplemented with 10% fetal bovine serum. Cells were washed with PBS and then incubated on ice in modified RIPA buffer, containing 150 mM sodium chloride, 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic acid , 0.1% SDS and 0.01% (w/v) sodium azide 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 uM Aprotinin, 5 uM Bestatin, 1.5 uM E-64, 2 uM Leupeptin Hemisulfate, 1 uM 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. Clone 15 Lysate Fractionation: Whole Cell Lysate Lysate Stimulation: Not Stimulated Culture Type: Tissue Culture
Gene ID	6091
Gene Symbol	ROBO1
Species	Human

Preparation Method	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, containing 150 mM sodium chloride, 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.5% sodium deoxycholic acid , 0.1% SDS and 0.01% (w/v) sodium azide 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 uM Aprotinin, 5 uM Bestatin, 1.5 uM E-64, 2 uM Leupeptin Hemisulfate, 1 uM 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.
Lysate Type	Knockout A549 Cell
Lysate Life Stage	Adult
Lysate Subcellular Fraction	Whole

Product Application Details

Applications	Western Blot, ELISA, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot, ELISA, Immunoprecipitation, SDS-Page, Chromatin Immunoprecipitation (ChIP)
Application Notes	This product 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.

Images

Western Blot: ROBO1 Knockout A549 Cell Lysate [NBP3-18631] - Lane 1: Opal Prestained MW Marker. 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. Lane 9: MCF-7 WCL. Load: 35ug lysate/lane. Primary Antibody [Blot A] Anti-ROBO1 ~181kDa; [Blot B] stripped and re-probed with Anti-Tubulin ~50kDa; at 1ug/mL overnight at 2-8C. 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.	
Western Blot of ROBO1 Knockout A549 Cell Lysate. Lane 1: Opal Prestained MW Marker	

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Products Related to NBP3-18631

NBP2-68981PEP	ROBO1 Recombinant Protein Antigen
DVE00	VEGF [HRP]
AF1749	ROBO1 Antibody
AF566	Neuropilin-1 Antibody [Unconjugated]

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis.
Lysates are guaranteed for 6 months from date of receipt.

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