

Datasheet for ABIN2774396
anti-ORMDL3 antibody (N-Term)[Go to Product page](#)

1 Validation

1 Image

Overview

Quantity:	100 µL
Target:	ORMDL3
Binding Specificity:	N-Term
Reactivity:	Human, Mouse, Rat, Guinea Pig, Horse, Rabbit, Zebrafish (Danio rerio), Cow, Dog, Goat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This ORMDL3 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Sequence:	GTAHSEVNPN TRVMNSRGIW LSYVLAIGLL HIVLLSIPFV SVPVWTLTN
Specificity:	Pan specific ORMDL1-3 antibody. Predicted to react with ORMDL1, ORMDL2 and ORMDL3.
Predicted Reactivity:	Cow: 100%, Dog: 100%, Goat: 100%, Guinea Pig: 100%, Horse: 100%, Human: 100%, Mouse: 100%, Rabbit: 100%, Rat: 100%, Zebrafish: 100%
Characteristics:	This is a rabbit polyclonal antibody against ORMDL3. It was validated on Western Blot.
Purification:	Affinity Purified

Target Details

Target:	ORMDL3
Alternative Name:	ORMDL3 (ORMDL3 Products)

Target Details

Background: ORMDL3 is a negative regulator of sphingolipid synthesis. ORMDL3 may indirectly regulate endoplasmic reticulum-mediated Ca(+2) signaling.

Alias Symbols: -

Protein Interaction Partner: UBC, APP, LNX1, ELAVL1, SPTLC1, EEF1A1,

Protein Size: 153

Molecular Weight: 17 kDa

Gene ID: 94103

NCBI Accession: [NM_139280](#), [NP_644809](#)

UniProt: [Q8N138](#)

Application Details

Application Notes: Optimal working dilutions should be determined experimentally by the investigator.

Comment: Antigen size: 153 AA

Restrictions: For Research Use only

Handling

Format: Liquid

Concentration: Lot specific

Buffer: Liquid. Purified antibody supplied in 1x PBS buffer with 0.09 % (w/v) sodium azide and 2 % sucrose.

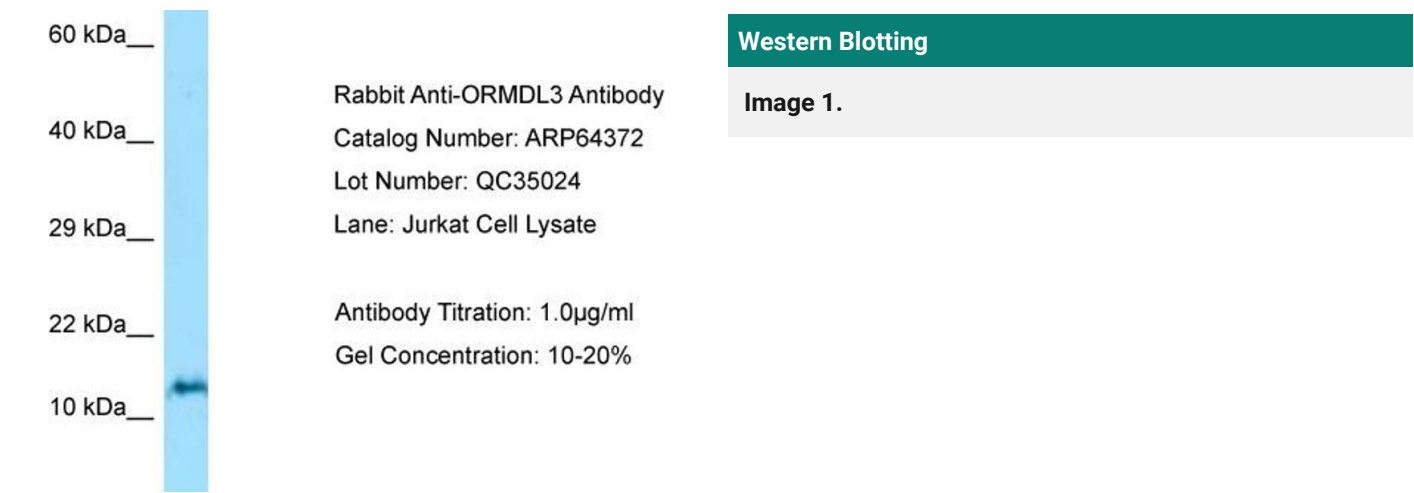
Preservative: Sodium azide

Precaution of Use: This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Handling Advice: Avoid repeated freeze-thaw cycles.

Storage: -20 °C

Storage Comment: For short term use, store at 2-8°C up to 1 week. For long term storage, store at -20°C in small aliquots to prevent freeze-thaw cycles.





Successfully validated (Western Blotting (WB))

by [Sita Devi Gupta Ph.D., Dr Teresa M Dunn Lab, Uniformed Services University of the Health Sciences](#)

Report Number: 103540

Date: Dec 03 2018

Target:	ORMDL3, 1, 2
Lot Number:	68904-43140
Method validated:	Western Blotting (WB)
Positive Control:	<p>Jurkat and HEK-293 cells for endogenous protein</p> <p>Flp-In T-Rex HEK-293 cells expressing Fusion SPT on induction with Tetracycline, which in turn induces endogenous ORMDLs. (https://www.ncbi.nlm.nih.gov/pubmed/25395622)</p> <p>Transient expression of NubG-HA ORMDL1, 2, or 3 in (PCR cloned from human gene expression library into yeast vector) HEK-293 cells</p> <p>Transient expression of ORMDL1-GFP in HEK-293 cells</p> <p>Jurkat cells stably expressing ORMDL3-HA generated using pUNO-ORMDL3-HA construct and blasticidine selection</p> <p>Purified ORMDL1/2/3-GFP</p>
Negative Control:	<p>siRNA against all the ORMDLs transfected together in Flp-In T-Rex HEK293 cells harboring Tet inducible Fusion serine palmitoyl transferase (LCB2a-ssSPTa-LCB1) SPT without and with Tet.</p> <p>siRNA from Dharmacon each one as ON-TARGET plus Human ORMDL1 (94101), human ORMDL2 (29095) and human ORMDL3 (94193) siRNA-SMARTpool.</p>
Notes:	Passed. ABIN2774396 specifically recognizes endogenous ORMDLs, transfected ORMDL1, 2, and 3 in HEK 293 cell lysates and purified ORMD1/2/3-GFP fusion proteins.
Primary Antibody:	ABIN2774396
Secondary Antibody:	goat anti-Rabbit IgG HRP conjugate (Bio-Rad, 170-6515, lot L005679 A)
Protocol:	<ul style="list-style-type: none"> 293T cells: <ul style="list-style-type: none"> Grow 293T (ATCC) or Flp-In T-Rex HEK-293 (Invitrogen) cells in DMEM (ThermoFisher Scientific, 10569044, lot 1929910) supplemented with 10% FBS (JRH Biosciences, 12103-78P, lot 4M2224) and penicillin-streptomycin (ThermoFisher Scientific, 15140122, lot 1864852) at 37°C and 5% CO₂ in 2ml on a 6 well dish to 60% confluency. Transfect 293T cells with 750ng NubG-HA-ORMDL1, 2, or 3 in pcDNA or ORMDL1-GFP fusion protein cloned into in pCMV6-ACGFP (Origene, RG202670) along with human LCB1,

2a and NubG-HA-ssSPTa using lipofectamine 2000 (ThermoFisher Scientific, 11668019, lot 1959316 or 1992885) following the manufacturer's instructions.

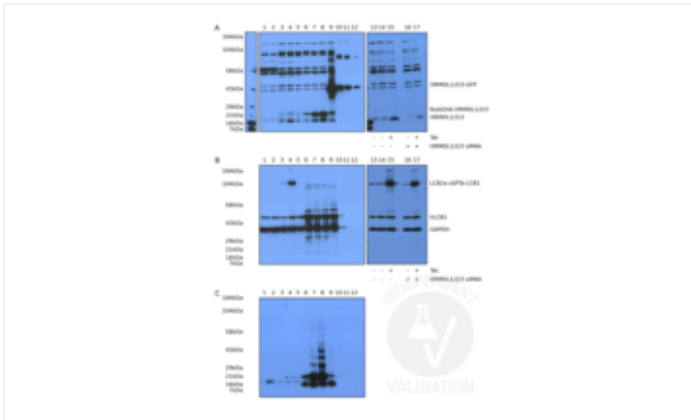
- Grow cells for 36h at 37°C and 5% CO₂.
- Jurkat cells:
 - Grow Jurkat Clone E6 (ATCC, TIB-152) and Jurkat cells stably expressing ORMDL3-HA RPMI-1640 Medium (ATCC,30-2001) supplemented with 10% FBS (JRH Biosciences, 12103-78P, lot 4M2224) at 37°C and 5% CO₂ in 2ml on a 6 well dish to 60% confluency.
- Flp-In T-Rex HEK-293:
 - Grow Flp-In T-Rex HEK-293 (Invitrogen) with tetracycline inducible fusion-SPT cells in DMEM (ThermoFisher Scientific, 10569044, lot 1929910) supplemented with 10% FBS (JRH Biosciences, 12103-78P, lot 4M2224) and penicillin-streptomycin (ThermoFisher Scientific, 15140122, lot 1864852) at 37°C and 5% CO₂ in 2ml on a 6 well dish to 60% confluency.
 - For tet-induction of SPT expression add 2µg/ml tetracycline.
 - For ORMDLs siRNA knockdown transfect a mix of 0.8ml optiMEM (each siRNA 7.5µl from 20µM stock diluted in 0.4ml optiMEM + 15µl lipofectamine in 0.4ml optiMEM) with a final concentration of 50nM of ORMDL1 (Dharmacon, 94101), ORMDL2 (Dharmacon, 29095), and ORMDL3 (Dharmacon, 94193) siRNA each into Flp-In T-Rex HEK293 cells (2.2ml optiMEM) harboring tet-inducible Fusion serine palmitoyl transferase (LCB2a-ssSPTa-LCB1) SPT using lipofectamine 2000 (ThermoFisher Scientific, 11668019, lot 1959316 or 1992885) following the manufacturer's instructions into a final volume of 3ml. After 10h incubation, add complete DMEM media with 2µg/ml tetracycline and incubate for 46h.
- Wash cells twice with ice-cold PBS.
- Lyse 1x10⁶ cells in 200-250µl per well cold lysis buffer: 2 volumes RIPA buffer (50mM Tris-HCl pH8, 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS and 1mM EDTA) and 1 volume membrane buffer (50mM Tris-HCl pH7.5, 1mM EGTA, 30 % glycerol), protease and phosphatase inhibitors.
- Determine total protein content of the lysates using Protein Assay Dye (Bio-Rad, 5000006, lot L007096 C).
- Denature about 200µg (35µg protein for Flp-In T-Rex HEK293 cells) of total protein for 14min at 70°C in 100µl NuPAGE LDS sample buffer (ThermoFisher Scientific, NP0008, lot 736752) and subsequently separate them on a 4-12% Bis-Tris Protein Gel (ThermoFisher Scientific, NP0336BOX, lot 18092071) at 150V for 10min and 185V for 40min.
- Transfer proteins onto nitrocellulose membrane (Bio-Rad, 1620213, lot 1603053) for 2h at 85V.
- Block membrane with TBST (20mM Tris-HCl, pH7.5, 150mM NaCl with 0.1% Tween 20) and 5% Blocking-Grade Blocker (Bio-Rad, 170-6404, lot L005668 A) for 1h at RT.
- Incubation with rabbit anti-ORMDL3 (antibodies-online, ABIN2774396, lot 68904-43140) diluted 1:1000 in TBST with 2% Blocking-Grade Blocker for 1h at RT.
- Wash membrane with deionized water.
- Wash membrane 3x for 10min with TBST.
- Incubation with secondary goat anti-Rabbit IgG HRP conjugate (Bio-Rad, 170-6515, lot L005679 A) diluted 1:5000 in TBST with 2% Blocking-Grade Blocker for 1h at RT.

- Wash membrane with deionized water and then 4x for 10min with TBST.
- Reveal protein bands using Western Lightning Plus-ECL (PerkinElmer, NEL105001EA, lot 203-17431) on a Kodak X-OMAT 2000A processor using Radiomat LS 7980052 films (MidSci).

Experimental Notes:

- ABIN2774396 reveals a protein of the expected molecular weight in lysates of 293T and in Flp-In T-Rex HEK293 cells harboring Tet inducible Fusion SPT upon Tet induction. It also showed the expected size protein band in HEK-293 cells transiently transfected with ORMDL1/2 or 3 tagged recombinant plasmids and with the purified ORMDL-GFPs (GFP fusion proteins run higher due to the tag).
- ABIN2774396 did not detect recombinant ORMDL3-HA in total lysates from Jurkat cells stably expressing ORMDL3-HA.
- ORMDL protein bands are visible in the positive samples and were significantly decreased upon knockdown using ORMDLs siRNAs. siRNA knockdown showed reduced expression of ORMDLs in uninduced and Tet induced fusion SPT cells as compared to the respective controls.
- In Jurkat cells the signal is very weak but longer exposure reveals the protein at the expected molecular weight. With 293T cells we see a stronger band just above 21kDa. This band was absent in Jurkat cells and in the purified proteins.

Image for Validation report #103540



Validation image no. 1 for anti-ORM1-Like 3 (ORMDL3) (N-Term) antibody (ABIN2774396)

A. Western blot analysis using ABIN2774396 of ORMDL1, ORMDL2 and ORMDL3 expression in total lysates from Jurkat cells (1), Jurkat cells stably expressing ORMDL3-HA (2), Flp-In T-Rex HEK293 cells with fusion SPT without and with tetracycline (3 and 4), 293T cells with pcDNA3.1 (5), HEK cells with NubG-HA tagged ORMDL1 (6), 2 (7) or 3 (8), transiently transfected with pCMVAC-ORMDL1-GFP (9), and purified ORMDL1-GFP (10), ORMDL2-GFP (11) and ORMDL3-GFP (12). ORMDLs siRNA expression decreases endogenous ORMDLs protein in uninduced cells and in Flp-In T-Rex HEK293 cells harboring tet inducible SPT as revealed by western blot using ABIN2774396 on lysates from Flp-In T-Rex HEK293 cells harboring Tet inducible SPT without Tet (13, 14) and with Tet (15) and all without siRNAs compared to cell lysates subsequently to siRNA ORMDL1, 2,

and 3 knockdown without (16) or with Tet induction (17). B. Western blot analysis of the same lysates using anti-human LCB1 and GAPDH antibodies and C. an anti-HA antibody.