Datasheet for ABIN654344

anti-ALX4 antibody (AA 249-275)

1 Validation

2 Images

Go to Product page

Overview

Quantity:	400 µL
Target:	ALX4
Binding Specificity:	AA 249-275
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This ALX4 antibody is un-conjugated
Application:	Western Blotting (WB), Flow Cytometry (FACS), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Immunogen:	This ALX4 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 249-275 amino acids from the Central region of human ALX4.
Clone:	RB28072
Isotype:	Ig Fraction
Predicted Reactivity:	B, M
Purification:	This antibody is purified through a protein A column, followed by peptide affinity purification.

Target Details

Target:	ALX4

Target Details

Alternative Name:	ALX4 (ALX4 Products)
Background:	This gene encodes a paired-like homeodomain transcription factor expressed in the mesenchyme of developing bones, limbs, hair, teeth, and mammary tissue. Mutations in this gene cause parietal foramina 2 (PFM2), an autosomal dominant disease characterized by deficient ossification of the parietal bones. Mutations in this gene also cause a form of frontonasal dysplasia with alopecia and hypogonadism, suggesting a role for this gene in craniofacial development, mesenchymal-epithelial communication, and hair follicle development. Deletion of a segment of chromosome 11 containing this gene, del(11)(p11p12), causes Potocki-Shaffer syndrome (PSS), a syndrome characterized by craniofacial anomalies, mental retardation, multiple exostoses, and genital abnormalities in males. In mouse, this gene has been shown to use dual translation initiation sites located 16 codons apart. [provided by RefSeq].
Molecular Weight:	44241
Gene ID:	60529
NCBI Accession:	NP_068745
UniProt:	Q9H161

Application Details

Application Notes:	WB 1:1000 FC 1:10-50 CUT&RUN 1:100
Restrictions:	For Research Use only

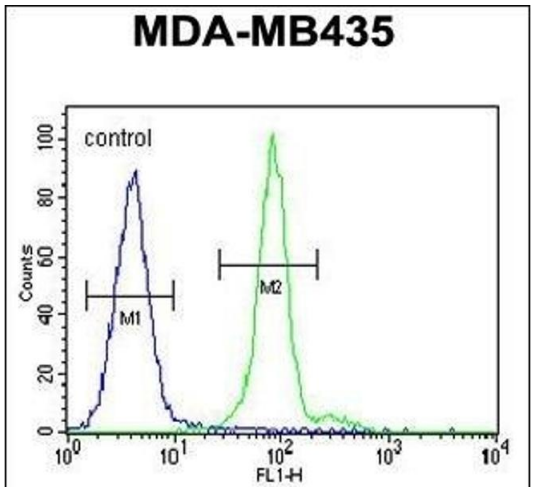
Handling

Format:	Liquid
Buffer:	Purified polyclonal antibody supplied in PBS with 0.09 % (W/V) sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Maintain refrigerated at 2-8 °C for up to 6 months. For long term storage store at -20 °C in small

Handling

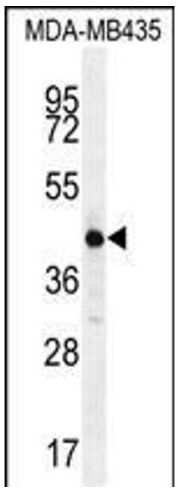
	aliquots to prevent freeze-thaw cycles.
Expiry Date:	6 months

Images



Flow Cytometry

Image 1. ALX4 Antibody (Center) (ABIN654344 and ABIN2844113) flow cytometric analysis of MDA-M cells (right histogram) compared to a negative control cell (left histogram).FITC-conjugated goat-anti-rabbit secondary antibodies were used for the analysis.



Western Blotting

Image 2. ALX4 Antibody (Center) (ABIN654344 and ABIN2844113) western blot analysis in MDA-M cell line lysates (35 µg/lane).This demonstrates the ALX4 antibody detected the ALX4 protein (arrow).

Successfully validated (Cleavage Under Targets and Release Using Nuclease (CUT&RUN))

by Gianluca Zambanini, Anna Nordin and Claudio Cantù; Cantù Lab, Gene Regulation during Development and Disease, Linköping University

Report Number: 104401

Date: Apr 26 2023

Target:	ALX4
Lot Number:	SA101101AA
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Polyclonal rabbit anti-H3K4me (antibodies-online, ABIN3023251)
Negative Control:	Polyclonal guinea pig anti-rabbit IgG (antibodies-online, ABIN101961)
Notes:	Passed. The anti-ALX4 antibody ABIN2844113 allows for CUT&RUN target profiling of ALX4 in mouse forelimb cells.
Primary Antibody:	ABIN2844113
Protocol:	• Cell harvest and nuclear extraction ◦ Dissect 3 Fore limbs (11.5 DAC) from RjOrl:SWISS embryos for each sample. ◦ Dissociate the tissue into single cells in TrypLE for 15 min at 37 °C. ◦ Centrifuge cell solution 5 min at 800 x g at RT. ◦ Remove the liquid carefully. ◦ Gently resuspend cells in 1 mL of Nuclear Extraction Buffer (20 mM HEPES-KOH pH 8.2, 20% Glycerol, 0,05% IGEPAL, 0.5 mM Spermidine, 10 mM KCl, Roche Complete Protease Inhibitor EDTA-free). ◦ Move the solution to a 2 mL centrifuge tube. ◦ Pellet the nuclei 800 x g for 5 min. ◦ Repeat the NE wash twice for a total of three washes. ◦ Resuspend the nuclei in 20 µL NE Buffer per sample. • Concanavalin A beads preparation ◦ Prepare one 2 mL microcentrifuge tube. ◦ Gently resuspend the magnetic Concanavalin A Beads (antibodies-online, ABIN6952467). ◦ Pipette 20 µL Con A Beads slurry for each sample into the 2 mL microcentrifuge tube. ◦ Place the tube on a magnet stand until the fluid is clear. Remove the liquid carefully. ◦ Remove the microcentrifuge tube from the magnetic stand. ◦ Pipette 1 mL Binding Buffer (20 mM HEPES pH 7.5, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$) into the tube and resuspend ConA beads by gentle pipetting. ◦ Spin down the liquid from the lid with a quick pulse in a table-top centrifuge. ◦ Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.

- Remove the microcentrifuge tube from the magnetic stand.
- Repeat the wash twice for a total of three washes.
- Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original volume of bead slurry, i.e. 20 µL per sample.

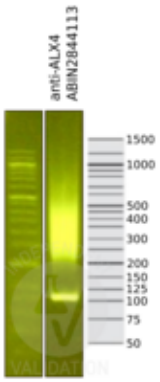
- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 1.5 mL tube on the magnet rack and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 1 mL of EDTA Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free, 2 mM EDTA).
  - Incubate for 5 min at RT.
  - Place the tube on the magnet stand and when the liquid is clear remove the supernatant.
  - Resuspend the beads in 200 µL of Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) per sample.
- Primary antibody binding
  - Divide nuclei suspension into separate 200 µL PCR tubes, one for each antibody (150,000 cells per sample).
  - Add 2 µL antibody (anti-ALX4 antibody ABIN2844113, anti-H3K4me positive control antibody ABIN3023251, guinea pig anti-rabbit IgG negative control antibody ABIN101961) to the respective tube, corresponding to a 1:100 dilution.
  - Incubate ON at 4 °C.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash buffer (to accelerate the process use a multichannel pipette).
  - Repeat the wash for a total of five washes.
- pAG-MNase Binding
  - Prepare a 1.5 mL microcentrifuge tube containing 200 µL of pAG mix pear sample (200 µL of wash buffer + 120 ng pAG-MNase per sample).
  - Place the PCR tubes with the sample on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove tubes from the magnetic stand.
  - Resuspend the beads in 200 µL of pAG-MNase premix.
  - Incubate for 30 min at 4 °C.
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Wash with 200 µL of Wash Buffer using a multichannel pipette to accelerate the process.
  - Repeat the wash for a total of five washes.
  - Resuspend in 200 µL of Wash Buffer.
- MNase digestion and release of pAG-MNase-antibody-chromatin complexes
  - Place PCR tubes on ice and allow to chill.
  - Prepare a 1.5 mL microcentrifuge tube with 51 µL of 2 mM $CaCl_2$ mix per sample (50 µL

Wash Buffer + 1 µL 100 mM $CaCl_2$) and let it chill on ice.
    - Always in ice, place the samples on the magnetic rack and when the liquid is clear remove the supernatant.
    - Resuspend the samples in 50 µL of the 2 mM CaCl2 mix and incubate in ice for exactly 30 min.
    - Place the sample on the magnet stand and when the liquid is clear move the supernatant in fresh collection tubes with 3 µL of EDTA/EGTA 0.25 M (Digestion buffer).
    - Resuspend the sample in 47 µL of 1x Urea STOP Buffer (8.5 M Urea, 100 mM NaCl, 2 mM EGTA, 2 mM EDTA, 0,5% IGEPAL).
    - Incubate the samples for 1 h at 4 °C.
    - Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to the previously collected digestion buffer.
- DNA Clean up
    - Take the Mag-Bind® TotalPure NGS beads (Omega Bio-Tek, M1378-01) from the storage and wait until they are RT.
    - Add 2x volume of beads to each sample (e.g. 100 µL of beads for 50 µL of sample).
    - Incubate the beads and the sample for 15 min at RT.
    - During incubation prepare fresh EtOH 80%.
    - Place the PCR tubes on a magnet stand and when the liquid is clear remove the supernatant.
    - Add 200 µl of fresh 80% EtOH to the sample without disturbing the.
    - Incubate 30 sec at RT.
    - Remove the EtOH from the sample.
    - Repeat the wash with 80% EtOH.
    - Resuspend the beads in 25 µL of 10 mM Tris.
    - Incubate the sample for 2 min at RT.
    - Repeat the 2x beads clean up as described before (this time with 50 µL of beads for each sample).
    - Resuspend the beads and DNA in 20 µL of 10 mM Tris.
- Library preparation and sequencing
    - Prepare Libraries using KAPA HyperPrep Kit using KAPA Dual-Indexed adapters according to protocol.
    - Sequence samples on an Illumina NextSeq 500 sequencer, using a NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 36 bp PE.
- Peak calling
    - Trim reads using using bbTools bbduk (BBMap - Bushnell B. - sourceforge.net/projects/bbmap/) to remove adapters, artifacts and repeat sequences.
    - Map aligned reads to the mm10 mouse genome using bowtie with options -m 1 -v 0 -l 0 -X 500.
    - Use SAMtools to convert SAM files to BAM files and remove duplicates.
    - Use BEDtools genomecov to produce Bedgraph files.
    - Call peaks using SEACR with a 0.001 threshold and the option norm stringent.

Experimental Notes:	The protocol is published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U)

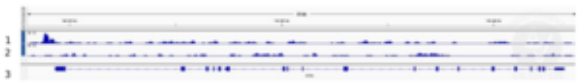
protocol uncovers Wnt/β-catenin tissue-specific genomic targets. Development (2022). PMID 36355069

Images for Validation report #104401



Validation image no. 1 for anti-ALX Homeobox 4 (ALX4) (AA 249-275) antibody (ABIN2844113)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using the anti-ALX4 antibody ABIN2844113 (right) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (left).



Validation image no. 2 for anti-ALX Homeobox 4 (ALX4) (AA 249-275) antibody (ABIN2844113)

1. Alignment tracks from CUT&RUN targeting ALX4 in mouse fore limb (11.5) cells using anti-ALX4 antibody ABIN2844113, showing the Eif3b locus. 2. Alignment tracks using negative control IgG, ABIN101961. 3. RefSeq Genes.