Datasheet for ABIN6147364

anti-SALL4 antibody (AA 1-220)

1 Validation | 2 Images | 1 Publication

Go to Product page

Overview

Quantity:	100 µL
Target:	SALL4
Binding Specificity:	AA 1-220
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This SALL4 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF)

Product Details

Immunogen:	Recombinant fusion protein containing a sequence corresponding to amino acids 1-220 of human SALL4 (NP_065169.1).
Sequence:	MSRRKQAKPQ HINSEEDQGE QQPQQQTPEF ADAAPAAPAA GELGAPVNHP GNDEVASEDE ATVKRLRREE THVCEKCCAE FFSISEFLEH KKNCTKNPPV LIMNDSEGPV PSEDFSGAVL SHQPTSPGSK DCHRENGGSS EDMKEKPDAE SVVYLKTETA LPPTPQDISY LAKGKVANTN VTLQALRGTK VAVNQRSADA LPAPVPGANS IPWVLEQILC
Isotype:	IgG
Cross-Reactivity:	Human, Mouse
Characteristics:	Polyclonal Antibodies

Target Details

Target:	SALL4
Alternative Name:	SALL4 (SALL4 Products)
Background:	This gene encodes a zinc finger transcription factor thought to play a role in the development of abducens motor neurons. Defects in this gene are a cause of Duane-radial ray syndrome (DRRS). Alternative splicing results in multiple transcript variants encoding different isoforms.,SALL4,DRRS,HSAL4,ZNF797,Epigenetics & Nuclear Signaling,Cell Biology & Developmental Biology,Stem Cells,Embryonic Stem Cells,SALL4
Molecular Weight:	65 kDa/112 kDa
Gene ID:	57167
UniProt:	Q9UJQ4
Pathways:	Stem Cell Maintenance, Tube Formation

Application Details

Application Notes:	WB,1:500 - 1:2000,IF,1:50 - 1:100
Restrictions:	For Research Use only

Handling

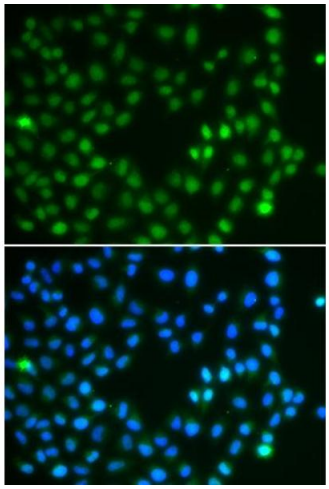
Format:	Liquid
Buffer:	PBS with 0.02 % sodium azide,50 % glycerol, pH 7.3.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store at -20°C. Avoid freeze / thaw cycles.

Publications

Product cited in:	Diener, Baggiolini, Pernebrink, Dalcher, Lerra, Cheng, Varum, Häusel, Stierli, Treier, Studer, Basler, Levesque, Dummer, Santoro, Cantù, Sommer: "Epigenetic control of melanoma cell invasiveness by the stem cell factor SALL4." in: **Nature communications**, Vol. 12, Issue 1, pp. 5056, (2021) (PubMed).

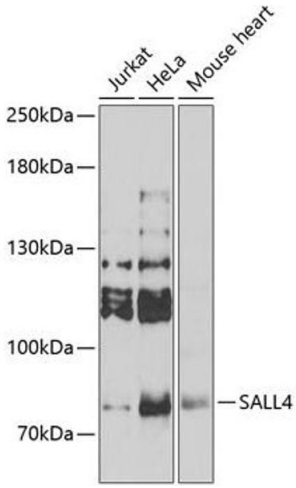
Publications

Images



Immunofluorescence

Image 1. Immunofluorescence analysis of A549 cells using SALL4 antibody (ABIN6132627). Blue: DAPI for nuclear staining.



Western Blotting

Image 2. Western blot analysis of extracts of various cell lines, using SALL4 Antibody (ABIN6132627) at 1:1000 dilution. Secondary antibody: HRP Goat Anti-Rabbit IgG (H+L) (ABIN3020597) at 1:10000 dilution. Lysates/proteins: 25ug per lane. Blocking buffer: 3% nonfat dry milk in TBST.

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

by Cantù Lab, Gene Regulation during Development and Disease, Linköping University

Report Number: 104300

Date: Aug 20 2021

Target:	SALL4
Lot Number:	29100201
Method validated:	Cleavage Under Targets and Release Using Nuclease (CUT&RUN)
Positive Control:	Recombinant anti-H3K27me3 CUT&RUN Positive Control antibody (antibodies-online, ABIN6923144)
Negative Control:	Monoclonal anti-FLAG (Sigma-Aldrich, F3165)
Notes:	Passed. ABIN6132627 allows for SALL4 targeted digestion of genomic DNA using CUT&RUN.
Primary Antibody:	ABIN6132627
Protocol:	• Cell harvest ○ Harvest 500,000 human melanoma cells per antibody to be used at RT. /li> ○ Centrifuge cell solution 3 min at 600 x g at RT. ○ Remove the liquid carefully. ○ Gently resuspend cells in 1 mL Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) by pipetting and transfer cell solution to a 2 mL microcentrifuge tube. ○ Centrifuge cell solution 3 min at 600 x g at RT and discard the supernatant. ○ Repeat twice for a total of three washes. ○ Resuspend cell pellet in 1 mL Wash Buffer by gently pipetting. • Concanavalin A beads preparation ○ Prepare one 1.5 mL microcentrifuge tube. ○ Gently resuspend the magnetic Concanavalin A Beads (antibodies-online, ABIN6923139). ○ Pipette 10 µL Con A Beads slurry for each sample into the 1.5 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 each 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 twice for a total of three washes.

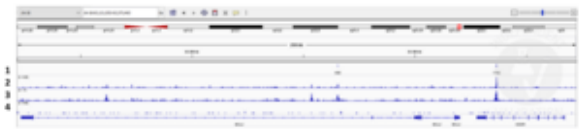
  - Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original volume of bead slurry, i.e. 10 µL per sample.
- Cell immobilization – binding to Concanavalin A beads
  - Carefully vortex the cell suspension and add 10 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly and rotate for 10 min at RT.
- Cell permeabilization and primary antibody binding
  - Divide cell suspension into separate 2 mL microcentrifuge tubes, one for each antibody (500,000 cells per sample).
  - Place the microcentrifuge tubes on a magnetic stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 150 µL Digitonin Wash buffer (wash buffer with 0.025% (wt/vol) Digitonin) supplemented with 2 mM EDTA.
  - Gently vortex the microcentrifuge tubes until the beads are resuspended.
  - Add 1.5 µL antibody (anti-SALL4 antibody ABIN6132627, anti-H3K27me3 positive control antibody ABIN6923144, and anti-FLAG tag antibody negative control) to the respective tube, corresponding to a 1:100 dilution.
  - Rotate the microcentrifuge tubes ON at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 ml pipette tip.
  - Repeat once for a total of two washes.
- pAG-MNase Binding
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Vortex the sample at low speed and add 3.75 µL 20X CUTANA pAG-MNase for ChIC/CUT&RUN Assays (ABIN6950951) to 0.5X in 150 µL Digitonin Wash Buffer per sample, gently resuspending the beads by pipetting.
  - Rotate the microcentrifuge tubes for 1 h at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 mL pipette tip.
  - Repeat once for a total of two washes.
- MNase digestion and release of pAG-MNase-antibody-chromatin complexes
  - 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.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 100 µL

Digitonin Wash buffer per sample along the side of the tube.
  - Place tubes in a heat block, kept on ice, and allow to chill.
  - Add 2 µL 0.1 M $CaCl_2$ to each sample.
  - Incubate tubes at 0 °C for 30 min.
  - Add 100 µL 2xSTOP buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.05% (wt/vol) Digitonin, 100 µg/mL RNAse A, 50 µg/mL Glycogen).
  - Incubate tubes at 37 °C for 30 min.
  - Place the tubes on a magnet stand until the fluid is clear.
  - Transfer the supernatant containing the pA-MNase-bound digested chromatin fragments to fresh 1.5 mL microcentrifuge tubes.
- DNA extraction
  - Add 2 µL 10% SDS to a final concentration of 0.1% and 2.5 µL Proteinase K (20 mg/mL) to each supernatant.
  - Gently vortex tubes at a low speed of approximately 1,100 rpm.
  - Incubate tubes at 50 °C for 1 h.
  - Add 200 µL PCI to tube.
  - Vortex tubes thoroughly at high speed until the liquid appears milky.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at RT for 5 min.
  - Carefully transfer to upper aqueous phase to a fresh 1.5 mL microcentrifuge tube containing 2 µL glycogen (diluted 1:10 to 2 mg/mL from the 20 mg/mL stock solution).
  - Add 20 µL 3 M NaOAc pH 5.2.
  - Add 400 µL 100% ethanol.
  - Place tubes for at -20 °C ON.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 5min.
  - Remove the liquid carefully with a pipette.
  - Wash pellet with 1ml 70% ethanol.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 1 min.
  - Remove the liquid carefully with a pipette.
  - Air-dry the pellet, then dissolve in 30 µL 1 mM Tris-HCl, 0.1 mM EDTA.
- 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), 36bp PE.
- Bioinformatics
  - Align reads the human genome (hg38) using bowtie78 with settings -X 700 -m1 -v 3. Remove duplicate reads, and sort files using samtools. Filter mapped reads for size, keeping only reads with a fragment size at or below 120 base pairs.
  - Generate bedgraph files using bedtools genomecov.
  - Call peaks using SEACR version 1.3, in relaxed mode, normalizing to the negative control.

Experimental Notes:	Results are published in Diener, J., Baggiolini, A., Pernebrink, M. et al. Epigenetic control of melanoma cell invasiveness by the stem cell factor SALL4. Nat Commun 12, 5056 (2021).

https://doi.org/10.1038/s41467-021-25326-8

Image for Validation report #104300



Validation image no. 1 for anti-Sal-Like 4 (SALL4) (AA 1-220) antibody (ABIN6132627)

Alignment tracks from CUT&RUN targeting SALL4 in human melanoma cells. 1. Peaks called by SEACR from CUT&RUN data using subnucleosomal reads. 2. Alignment track for subnucleosomal CUT&RUN reads obtained using anti-SALL4 antibody ABIN6132627 in human melanoma cells. 3. Alignment track for all reads obtained through CUT&RUN using anti-SALL4 antibody ABIN6132627 in human melanoma cells. 4. Alignment track for negative control.