Datasheet for ABIN6991990

anti-SMARCA4 antibody (C-Term)

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Overview

Quantity:	0.1 mg
Target:	SMARCA4
Binding Specificity:	AA 1420-1470, C-Term
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This SMARCA4 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunofluorescence (IF), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Immunogen:	SMARCA4 antibody was raised against a 19 amino acid peptide near the carboxy terminus of human SMARCA4. The immunogen is located within amino acids 1420 - 1470 of SMARCA4.
Isotype:	IgG
Specificity:	SMARCA4 antibody is human, mouse and rat reactive. Multiple isoforms of SMARCA4 are known to exist.
Purification:	SMARCA4 antibody is affinity chromatography purified via peptide column.

Target Details

Target:	SMARCA4

Target Details

Alternative Name:	SMARCA4 (SMARCA4 Products)
Background:	The SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily a member 4 (SMARCA4) protein, also known as BRG1, is a member of the SWI/SNF family of proteins and is similar to the brahma protein of Drosophila (1). Members of this family have helicase and ATPase activities and are thought to regulate transcription of certain genes by altering the chromatin structure around those genes (2). The encoded protein is part of the large ATP-dependent chromatin remodeling complex SNF/SWI, which is required for transcriptional activation of genes normally repressed by chromatin (3). In addition, this protein can bind BRCA1, as well as regulate the expression of the tumorigenic protein CD44 (4,5).
Molecular Weight:	Predicted: 185 kDa Observed: 200 kDa
Gene ID:	6597
NCBI Accession:	NP_001122321
UniProt:	P51532
Pathways:	Intracellular Steroid Hormone Receptor Signaling Pathway, Regulation of Intracellular Steroid Hormone Receptor Signaling, Stem Cell Maintenance

Application Details

Application Notes:	The rabbit anti-SMARCA4 antibody ABIN6991990 is suitable for use in CUT&RUN, immunofluorescence, immunohistochemistry, immunofluorescence, and Western Blot. Specific conditions for each assay should be optimized by the end user. General dilution recommendations for different applications are as follows: IHC: 1:500 IF: 1:50 WB: 1:500-1:1,000 CUT&RUN: 1:100
Restrictions:	For Research Use only

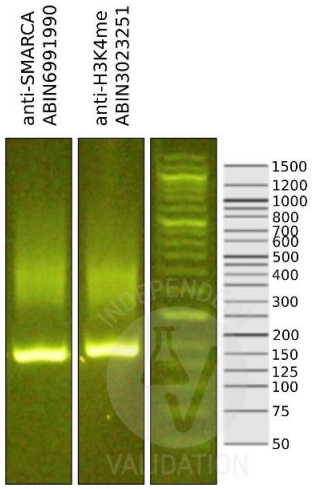
Handling

Format:	Liquid
Concentration:	1 mg/mL

Handling

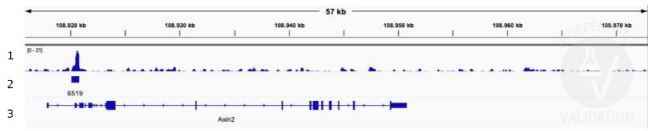
Buffer:	SMARCA4 Antibody is supplied in PBS containing 0.02 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C,4 °C
Storage Comment:	SMARCA4 antibody can be stored at 4°C for three months and -20°C, stable for up to one year.

Images



Cleavage Under Targets and Release Using Nuclease

Image 1. Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-SMARCA 4 antibody ABIN6991990 and anti-H3K4me antibody ABIN3023251 after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher). Images provided by Gianluca Zambanini, Anna Nordin and Claudio Cantù, Gene Regulation during Development and Disease, Linköping University (https://liu.se/en/research/cantu-lab).



Cleavage Under Targets and Release Using Nuclease

Image 2. Alignment tracks from CUT&RUN targeting SMARCA4 in Mouse froe limb (11.5) cells using anti-SMARCA4 antibody ABIN6991990 (1). Peaks called by SEACR from CUT&RUN data using anti-SMARCA4 antibody ABIN6991990 (2). RefSeq Genes (3). Images provided by Gianluca Zambanini, Anna Nordin and Claudio Cantù, Gene Regulation during Development and Disease, Linköping University (https://liu.se/en/research/cantu-lab).

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: 104416

Date: Feb 28 2022

Target:	SMARCA4
Lot Number:	7749-1303
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. ABIN6991990 allows for SMARCA4 targeted digestion using CUT&RUN in mouse fore limb (11.5) cells.
Primary Antibody:	ABIN6991990
Protocol:	• Cell harvest and nuclear extraction ◦ Dissect 3 Fore limbs (11.5 DAC) from mouse strain RjOrl:SWISS 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.

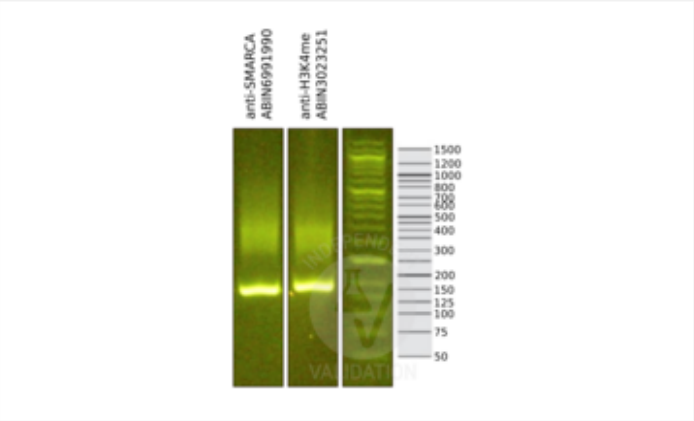
- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 2 mL tube on the magnet stand and when the liquid 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, 2mM EDTA).
  - Incubate 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.
  - Add 2 µL antibody (anti-SMARCA4 antibody ABIN6991990, anti-H3K27me3 antibody positive control ABIN6923144, and guinea pig anti-rabbit IgG negative control antibody ABIN101961) to the respective tube, corresponding to a 1:100 dilution.
  - Incubate at 4 °C ON.
  - 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 five times for a total of six washes.
- pAG-MNase Binding
  - Prepare a 1.5 mL microcentrifuge tube containing 100 µL of pAG mix per sample (100 µL of wash buffer + 58.5 µg 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 100 µL of pAG-MNase premix.
  - Incubate 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 five times for a total of six washes.
  - Resuspend in 100 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 102 µl of 2 mM $CaCl_2$ mix per sample (100 µl Wash Buffer + 2 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 100 µl of the 2 mM $CaCl_2$ mix and incubate in ice for exactly 30 min.
- Place the sample on the magnet stand and when the liquid is clear remove the supernatant.
- Resuspend the sample in 50 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 1h at 4°C.
- Transfer the supernatant containing the pAG-MNase-bound digested chromatin fragments to fresh 200 µl PCR tubes.

- DNA Clean up
  - Take the Mag-Bind® TotalPure NGS beads (Omega Bio-Tek, M1378-01) from the storage and wait until they are at 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 beads (Important!!! Do NOT resuspend the beads or remove the tubes from the magnet stand or the sample will be lost).
  - 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 + 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 hg38 human 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.

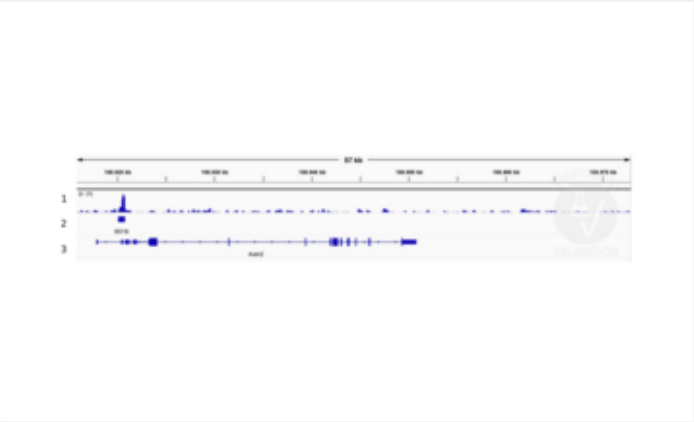
Experimental Notes:	The protocol is published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific genomic targets. bioRxiv (2022). https://doi.org/10.1101/2022.07.06.498999

Images for Validation report #104416



Validation image no. 1 for anti-SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4 (SMARCA4) (AA 1420-1470), (C-Term) antibody (ABIN6991990)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-SMARCA 4 antibody ABIN6991990 and anti-H3K4me antibody ABIN3023251 after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher).



Validation image no. 2 for anti-SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4 (SMARCA4) (AA 1420-1470), (C-Term) antibody (ABIN6991990)

1. Alignment tracks from CUT&RUN targeting SMARCA4 in Mouse froe limb (11.5) cells using anti-SMARCA4 antibody ABIN6991990. 2. Peaks called by SEACR from CUT&RUN data using anti-SMARCA4 antibody ABIN6991990. 3. RefSeq Genes.