Datasheet for ABIN5663810

anti-LEF1 antibody (AA 100-399)

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Overview

Quantity:	100 µL
Target:	LEF1
Binding Specificity:	AA 100-399
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This LEF1 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	Recombinant fusion protein containing a sequence corresponding to amino acids 100-399 of human LEF1 (NP_057353.1).
Sequence:	GLYNKGPSYS SYSGYIMMPN MNNDPYMSNG SLSPPIPRTS NKVPVVQPSH AVHPLTPLIT YSDEHFSPGS HPSHIPSDVN SKQGMSRHPP APDIPTFYPL SPGGVGQITP PLGWQGQPVY PITGGFRQPY PSSLSVDTSM SRFSHHMIPG PPGPHTTGIP HPAIVTPQVK QEHPHTDSDL MHVKPQHEQR KEQEPKRPHI KKPLNAFMLY MKEMRANVVA ECTLKESAAI NQILGRRWHA LSREEQAKYY ELARKERQLH MQLYPGWSAR DNYGKKKKRK REKLQESASG TGPRMTAAYI
Isotype:	IgG
Cross-Reactivity:	Human, Mouse, Rat
Characteristics:	Polyclonal Antibodies

Target Details

Target:	LEF1
Alternative Name:	LEF1 (LEF1 Products)
Background:	This gene encodes a transcription factor belonging to a family of proteins that share homology with the high mobility group protein-1. The protein encoded by this gene can bind to a functionally important site in the T-cell receptor-alpha enhancer, thereby conferring maximal enhancer activity. This transcription factor is involved in the Wnt signaling pathway, and it may function in hair cell differentiation and follicle morphogenesis. Mutations in this gene have been found in somatic sebaceous tumors. This gene has also been linked to other cancers, including androgen-independent prostate cancer. Alternative splicing results in multiple transcript variants.,LEF1,LEF-1,TCF10,TCF1ALPHA,TCF7L3,Epigenetics & Nuclear Signaling,Transcription Factors,Cancer,Tumor suppressors,Cell Biology & Developmental Biology,Cell Adhesion,Wnt/β-Catenin Signaling Pathway,Stem Cells,LEF1
Molecular Weight:	23 kDa/31 kDa/34 kDa/35 kDa/41 kDa/42 kDa/44 kDa
Gene ID:	51176
UniProt:	Q9UJU2
Pathways:	WNT Signaling, Intracellular Steroid Hormone Receptor Signaling Pathway, Regulation of Hormone Metabolic Process, Nuclear Hormone Receptor Binding, Chromatin Binding

Application Details

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

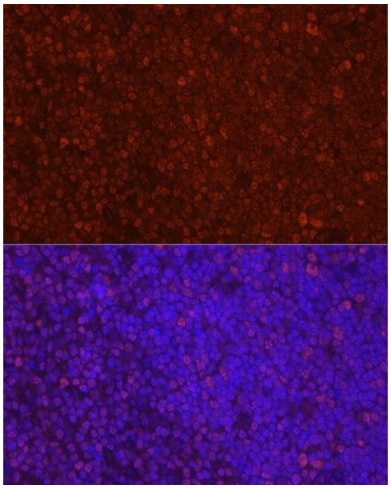
Handling

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

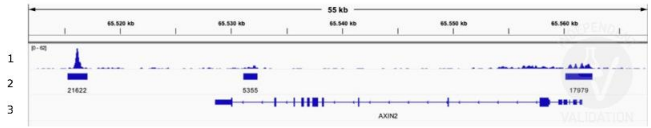
Product cited in:	Zambanini, Nordin, Jonasson, Pagella, Cantù: "A new cut&run low volume-urea (LoV-U) protocol optimized for transcriptional co-factors uncovers Wnt/b-catenin tissue-specific genomic targets." in: **Development (Cambridge, England)**, (2022) (PubMed).

Images



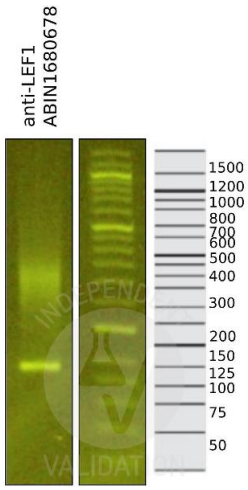
Immunofluorescence

Image 1. Immunofluorescence analysis of rat thymus cells using LEF1 Rabbit pAb (ABIN1680678, ABIN5663809, ABIN5663811 and ABIN6214021) at dilution of 1:25 (40x lens). Blue: DAPI for nuclear staining.



Cleavage Under Targets and Release Using Nuclease

Image 2. Alignment tracks from CUT&RUN targeting LEF1 in HEK293T cells using anti-LEF1 antibody ABIN1680678 (1). Peaks called by SEACR from CUT&RUN data using anti-LEF antibody ABIN1680678 (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).



Cleavage Under Targets and Release Using Nuclease

Image 3. Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using a LEF1 antibody (ABIN1680678) 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).

Images

Please check the product details page for more images. Overall 6 images are available for ABIN5663810.

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: 104350

Date: Feb 28 2022

Target:	LEF1
Lot Number:	3180201
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:	Polyclonal guinea Pig anti-rabbit IgG (antibodies-online, ABIN101961)
Notes:	Passed. ABIN1680678 allows for LEF1 targeted digestion using CUT&RUN in human HEK293T cells.
Primary Antibody:	ABIN1680678

Protocol:

- Cell harvest and nuclear extraction
  - Harvest 250,000 HEK293T cells per antibody to be used at RT stimulated with 10 µM CHIR for 24 h at RT.
  - Centrifuge cell solution 5 min at 600 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.

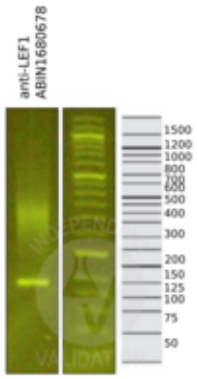
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tube from the magnetic stand.
  - Repeat the wash twice for a total of three washes.
  - Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original 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-LEF1 antibody ABIN1680678, 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.

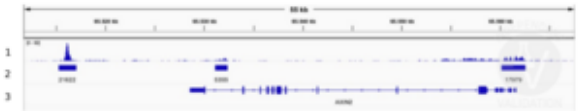
Experimental Notes:	Results are published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific genomic targets. bioRxiv (2022). https://doi.org/10.1101/2022.07.06.498999

Images for Validation report #104350



Validation image no. 1 for anti-Lymphoid Enhancer-Binding Factor 1 (LEF1) (AA 100-399) antibody (ABIN1680678)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-LEF1 antibody (ABIN1680678) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher).



Validation image no. 2 for anti-Lymphoid Enhancer-Binding Factor 1 (LEF1) (AA 100-399) antibody (ABIN1680678)

1. Alignment tracks from CUT&RUN targeting LEF1 in HEK293T cells using anti-LEF1 antibody ABIN1680678. 2 Peaks called by SEACR from CUT&RUN data using anti-LEF antibody ABIN1680678. 3. RefSeq Genes.