Datasheet for ABIN5619487

anti-DLX5 antibody (Center)

1 Validation

Go to Product page

Overview

Quantity:	100 µL
Target:	DLX5
Binding Specificity:	Center
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This DLX5 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC), Immunofluorescence (IF), Immunocytochemistry (ICC), Immunoprecipitation (IP), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Immunogen:	DLX5 antibody was raised in Rabbit using a KLH-conjugated synthetic peptide encompassing a sequence within the center region of human DLX5 as the immunogen
Specificity:	Recognizes endogenous levels of DLX5 protein
Cross-Reactivity (Details):	Mouse, Rat, Chicken
Characteristics:	Purified Polyclonal DLX5 antibody
Purification:	DLX5 antibody was purified by immunogen affinity chromatography

Target Details

Target:	DLX5

Target Details

Alternative Name:	DLX5 (DLX5 Products)

Application Details

Application Notes:	WB: 1:500 - 1:1000, IHC: 1:100 - 1:200, IF: 1:100 - 1:500, ICC: 1:100 - 1:500, IP: 1:10 - 1:100
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Supplied in liquid form in 0.42 % Potassium phosphate, 0.87 % Sodium chloride, pH 7.3 with 30 % glycerol and 0.01 % 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:	Store at 4 deg C for short term storage. For long term, aliquot and store at -20 deg C. Avoid repeat freeze/thaw cycles

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: 104402

Date: Dec 07 2022

Target:	DLX5
Lot Number:	X21080310
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. ABIN5619487 allows for CUT&RUN targeted profiling of DLX5 in mouse forelimb tissue.
Primary Antibody:	ABIN5619487
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 (Thermo Fisher Scientific) 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 (150,000 cells per sample).
  - Add 2 µL antibody (anti-DLX5 antibody ABIN5619487, anti-H3K4me positive control antibody ABIN3023251, 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.

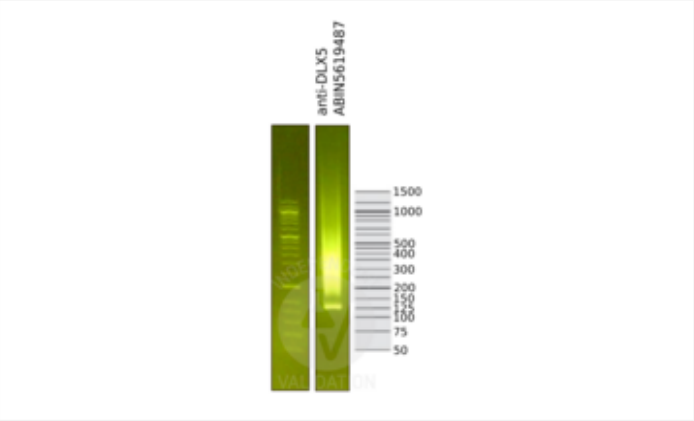
- Prepare a 1.5 mL microcentrifuge tube with 102 µl of 2 mM $CaCl_2$ mix per sample (100 µl Wash Buffer + 2 µL 100 mM $CaCl_2$) and let it chill on ice.
- Always in ice, place the samples on the magnetic rack and when the liquid is clear remove the supernatant.
- Resuspend the samples in 100 µl of the 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-HCl pH 8.2.
  - 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-HCl pH 8.2.
- 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.

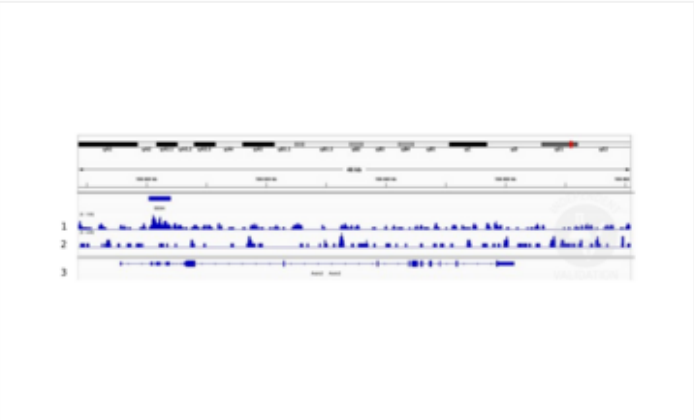
Experimental Notes:	The protocol is published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific genomic targets. Development (2022). PMID 36355069

Images for Validation report #104402



Validation image no. 1 for anti-Distal-Less Homeobox 5 (DLX5) (Center) antibody (ABIN5619487)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-DLX5 antibody ABIN5619487 (right) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (left).



Validation image no. 2 for anti-Distal-Less Homeobox 5 (DLX5) (Center) antibody (ABIN5619487)

1. Peaks for DLX5 called by SEACR. 2. Alignment tracks from CUT&RUN targeting DLX5 in mouse fore limb (11.5) cells using ABIN5619487, showing the Axin2 locus. 3. Alignment tracks using negative control IgG, ABIN1019613. 4. RefSeq Genes.