Datasheet for ABIN1386749

anti-HOXA13 antibody (AA 332-388)

1 Validation

1 Image

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Overview

Quantity:	100 µL
Target:	HOXA13
Binding Specificity:	AA 332-388
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This HOXA13 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunofluorescence (Cultured Cells) (IF (cc)), Immunofluorescence (Paraffin-embedded Sections) (IF (p)), Immunohistochemistry (Frozen Sections) (IHC (fro)), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunocytochemistry (ICC)

Product Details

Immunogen:	KLH conjugated synthetic peptide derived from human HOXA13
Isotype:	IgG
Cross-Reactivity:	Human
Predicted Reactivity:	Mouse,Rat,Cow,Sheep,Pig,Horse,Chicken,Rabbit
Purification:	Purified by Protein A.

Target Details

Target:	HOXA13

Target Details

Alternative Name:	HOXA13 (HOXA13 Products)
Background:	Synonyms: HOX1, HOX1J, Homeobox protein Hox-A13, Homeobox protein Hox-1J, HOXA13 Background: Sequence-specific, AT-rich binding transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis. Sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis.
Gene ID:	3209
UniProt:	P31271

Application Details

Application Notes:	WB 1:300-5000
	ELISA 1:500-1000
	IHC-P 1:200-400
	IHC-F 1:100-500
	IF(IHC-P) 1:50-200
	IF(IHC-F) 1:50-200
	IF(ICC) 1:50-200
	ICC 1:100-500
	CUT&RUN 1:100

Restrictions:	For Research Use only

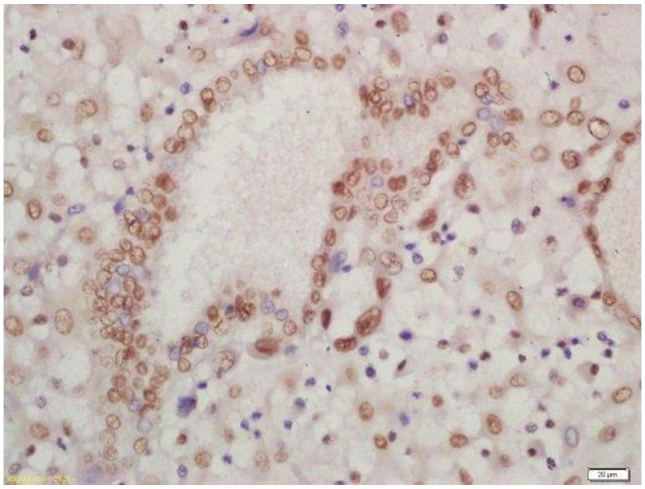
Handling

Format:	Liquid
Concentration:	1 μg/μL
Buffer:	0.01M TBS(pH 7.4) with 1 % BSA, 0.02 % Proclin300 and 50 % Glycerol.
Preservative:	ProClin
Precaution of Use:	This product contains ProClin: a POISONOUS AND HAZARDOUS SUBSTANCE, which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.

Handling

Expiry Date:	12 months

Images



Immunohistochemistry (Paraffin-embedded Sections)

Image 1. Formalin-fixed and paraffin embedded human placenta labeled with Rabbit Anti-HOXA13 Polyclonal Antibody, Unconjugated at 1:200 followed by conjugation to the secondary antibody and DAB staining

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: 104368

Date: Apr 26 2023

Target:	HOXA13
Lot Number:	AD02263837
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)
Primary Antibody:	ABIN1386749

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

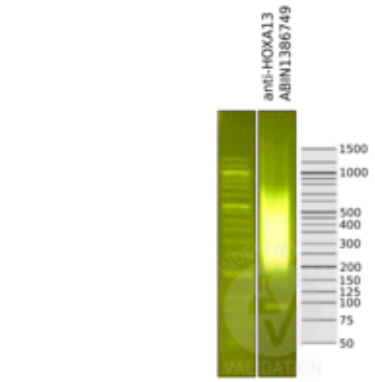
original volume of bead slurry, i.e. 20 µL per sample.

- 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-HOXA13 antibody ABIN1386749, 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.

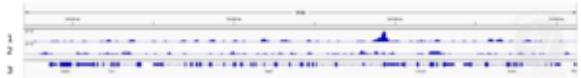
- DNA Clean up
  - Take the Mag-Bind® TotalPure NGS beads (Omega Bio-Tek, M1378-01) from the storage and wait until they are RT.
  - Add 2x volume of beads to each sample (e.g. 100 µL of beads for 50 µL of sample).
  - Incubate the beads and the sample for 15 min at RT.
  - During incubation prepare 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



Validation image no. 1 for anti-Homeobox A13 (HOXA13) (AA 332-388) antibody (ABIN1386749)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-HOXA13 antibody ABIN1386749 (right) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (left).



Validation image no. 2 for anti-Homeobox A13 (HOXA13) (AA 332-388) antibody (ABIN1386749)

1. Alignment tracks from CUT&RUN targeting HOXA13 in mouse fore limb (11.5 DAC) cells using anti-HOXA13 antibody ABIN1386749, showing the Usp5 locus. 2. Alignment tracks using negative control IgG, ABIN101961. 3. RefSeq Genes.