Datasheet for ABIN1387620

anti-FOXO1 antibody (AA 165-270)

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Overview

Quantity:	100 µL
Target:	FOXO1
Binding Specificity:	AA 165-270
Reactivity:	Human, Mouse, Rat, Cow, Pig, Dog, Chicken
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This FOXO1 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunofluorescence (Paraffin-embedded Sections) (IF (p)), Flow Cytometry (FACS)

Product Details

Immunogen:	KLH conjugated synthetic peptide derived from human FOXO1A
Isotype:	IgG
Cross-Reactivity:	Chicken, Cow, Dog, Human, Mouse, Pig, Rat
Purification:	Purified by Protein A.

Target Details

Target:	FOXO1
Alternative Name:	FOXO1A (FOXO1 Products)
Background:	Synonyms: Forkhead box protein O1, Forkhead box protein O1A, Forkhead in

Target Details

	rhabdomyosarcoma, FOXO1, FKHR, FOXO1A Background: Transcription factor that is the main target of insulin signaling and regulates metabolic homeostasis in response to oxidative stress. Binds to the insulin response element (IRE) with consensus sequence 5'-TT[G/A]TTTTG-3' and the related Daf-16 family binding element (DBE) with consensus sequence 5'-TT[G/A]TTTAC-3'. Activity suppressed by insulin. Main regulator of redox balance and osteoblast numbers and controls bone mass. Orchestrates the endocrine function of the skeleton in regulating glucose metabolism. Acts syngernistically with ATF4 to suppress osteocalcin/BGLAP activity, increasing glucose levels and triggering glucose intolerance and insulin insensitivity. Also suppresses the transcriptional activity of RUNX2, an upstream activator of osteocalcin/BGLAP. In hepatocytes, promotes gluconeogenesis by acting together with PPARGC1A to activate the expression of genes such as IGFBP1, G6PC and PPCK1. Important regulator of cell death acting downstream of CDK1, PKB/AKT1 and SKT4/MST1. Promotes neural cell death. Mediates insulin action on adipose. Regulates the expression of adipogenic genes such as PPARG during preadipocyte differentiation and, adipocyte size and adipose tissue-specific gene expression in response to excessive calorie intake. Regulates the transcriptional activity of GADD45A and repair of nitric oxide-damaged DNA in beta-cells.
Molecular Weight:	72kDa
Gene ID:	2308
Pathways:	PI3K-Akt Signaling, Cell Division Cycle, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Carbohydrate Homeostasis, Chromatin Binding, Regulation of Carbohydrate Metabolic Process, CXCR4-mediated Signaling Events, BCR Signaling

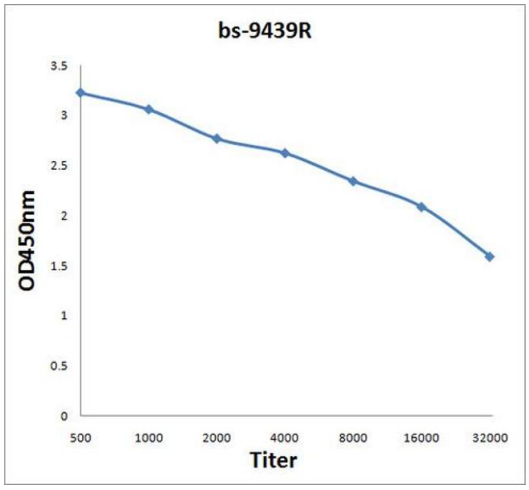
Application Details

Application Notes:	WB 1:300-5000 ELISA 1:500-1000 FCM 1:20-100 IHC-P 1:200-400 IF(IHC-P) 1:50-200 CUT&RUN 1:100
Restrictions:	For Research Use only

Handling

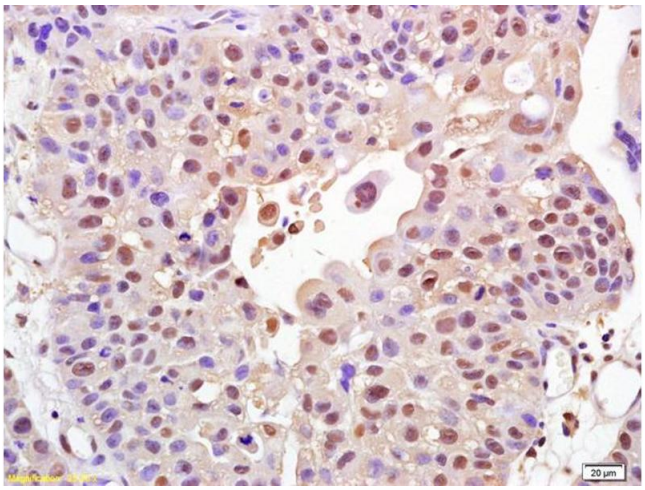
Format:	Liquid
Concentration:	1 μg/μL
Buffer:	0.01M TBS(pH 7.4) with 1 % BSA, 0.02 % Proclin300 and 50 % Glycerol.
Preservative:	ProClin
Precaution of Use:	This product contains ProClin: a POISONOUS AND HAZARDOUS SUBSTANCE, which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.
Expiry Date:	12 months

Validation report #104385 for Cleavage Under Targets and Release Using Nuclease (CUT&RUN)



ELISA

Image 1. Antigen: 0.2 μg/100 μL Primary: Antiserum, 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000; Secondary: HRP conjugated Goat-Anti-Rabbit IgG at 1: 5000; TMB staining; Read the data in MicroplateReader by 450



Immunohistochemistry

Image 2. Formalin-fixed and paraffin embedded human bladder carcinoma labeled with Rabbit Anti FOXO1A Polyclonal Antibody, Unconjugated (ABIN1387620) 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: 104385

Date: Apr 26 2023

Target:	FOXO1
Lot Number:	BA06042267
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. The anti-FOXO1 antibody ABIN1387620 allows for CUT&RUN targeting profiling of FOXO1 in mouse forelimb cells.
Primary Antibody:	ABIN1387620

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.

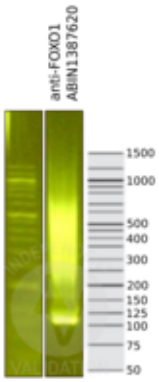
- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 1.5 mL tube on the magnet rack and when the liquid 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-FOXO1 antibody ABIN1387620, 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)

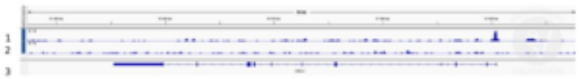
protocol uncovers Wnt/β-catenin tissue-specific genomic targets. Development (2022). PMID 36355069

Images for Validation report #104385



Validation image no. 1 for anti-Forkhead Box O1 (FOXO1) (AA 165-270) antibody (ABIN1387620)

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using anti-FOXO1 antibody ABIN1387620 (right) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (left).



Validation image no. 2 for anti-Forkhead Box O1 (FOXO1) (AA 165-270) antibody (ABIN1387620)

1. Alignment tracks from CUT&RUN targeting FOXO1 in mouse fore limb (11.5) cells using anti-FOXO1 antibody ABIN1387620, showing the Gtf2a1 locus. 2. Alignment tracks using negative control IgG, ABIN101961. 3. RefSeq Genes.