Datasheet for ABIN6265491

anti-TBX3 antibody

1 Validation

3 Images

Go to Product page

Overview

Quantity:	100 µL
Target:	TBX3
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This TBX3 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunocytochemistry (ICC), Immunofluorescence (IF), Cleavage Under Targets and Release Using Nuclease (CUT&RUN)

Product Details

Immunogen:	A synthesized peptide derived from human TBX3
Isotype:	IgG
Specificity:	TBX3 antibody detects endogenous levels of total TBX3
Cross-Reactivity:	Human, Mouse (Murine), Rat (Rattus)
Purification:	The antiserum was purified by peptide affinity chromatography using SulfoLink™ Coupling Resin (Thermo Fisher Scientific).

Target Details

Target:	TBX3
Alternative Name:	TBX3 (TBX3 Products)

Target Details

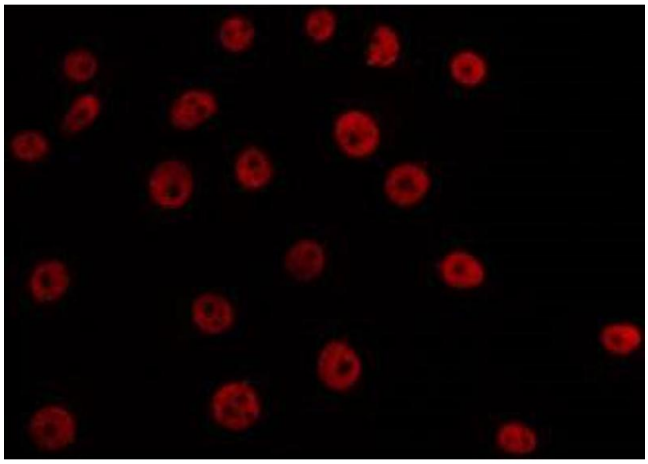
Background:	Description: Transcriptional repressor involved in developmental processes. Probably plays a role in limb pattern formation. Acts as a negative regulator of PML function in cellular senescence. Gene: TBX3
Molecular Weight:	79kDa
Gene ID:	6926
UniProt:	O15119, Q13207
Pathways:	Hormone Transport, Stem Cell Maintenance, Regulation of Muscle Cell Differentiation, Skeletal Muscle Fiber Development

Application Details

Application Notes:	WB: 1:500~1:3000, IF/ICC 1:100-1:500
Restrictions:	For Research Use only

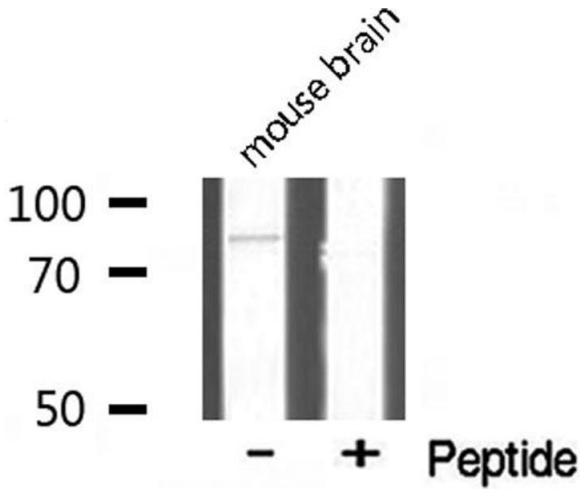
Handling

Format:	Liquid
Concentration:	1 mg/mL
Buffer:	Rabbit IgG in phosphate buffered saline , pH 7.4, 150 mM NaCl, 0.02 % sodium azide and 50 % glycerol.
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.Stable for 12 months from date of receipt
Expiry Date:	12 months



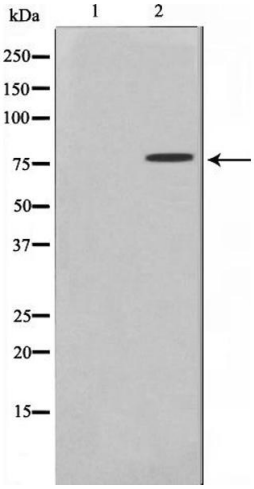
Immunofluorescence (fixed cells)

Image 1. ABIN6266698 staining A549 by IF/ICC. The sample were fixed with PFA and permeabilized in 0.1% Triton X-100,then blocked in 10% serum for 45 minutes at 25°C. The primary antibody was diluted at 1/200 and incubated with the sample for 1 hour at 37°C. An Alexa Fluor 594 conjugated goat anti-rabbit IgG (H+L) Ab, diluted at 1/600, was used as the secondary antibody.



Western Blotting

Image 2. Western blot analysis of TBX3 expression in mouse brain lysate



Western Blotting

Image 3. Western blot analysis on A549 cell lysate using TBX3 Antibody.The lane on the left is treated with the antigen-specific peptide.

Successfully validated (Cleavage Under Targets and Release Using Nuclease (CUT&RUN))

by Mattias Pernebrink and Claudio Cantù; Cantù Lab, Gene Regulation during Development and Disease, Linköping University

Report Number: 104234

Date: Mar 01 2021

Target:	TBX3
Lot Number:	10H2885
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:	Monoclonal anti-FLAG (Sigma-Aldrich, F3165)
Notes:	Passed. ABIN6265491 allows for TBX3 targeted digestion using CUT&RUN.
Primary Antibody:	ABIN6265491

Protocol:

- Cell harvest
  - Harvest cells from day 10.5 mouse embryo front limbs, estimating 90,000 cells per antibody to be used at RT.
  - Centrifuge cell solution 3 min at 600 x g at RT.
  - Remove the liquid carefully.
  - Gently resuspend cells in 1 mL Wash Buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, Roche Complete Protease Inhibitor EDTA-free) by pipetting and transfer cell solution to a 2 mL microcentrifuge tube.
  - Centrifuge cell solution 3 min at 600 x g at RT and discard the supernatant.
  - Repeat twice for a total of three washes.
  - Resuspend cell pellet in 1 mL Wash Buffer by gently pipetting.
- Concanavalin A beads preparation
  - Prepare one 1.5 mL microcentrifuge tube.
  - Gently resuspend the magnetic Concanavalin A Beads (antibodies-online, ABIN6923139).
  - Pipette 10 µL Con A Beads slurry for each sample into the 1.5 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 each 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.

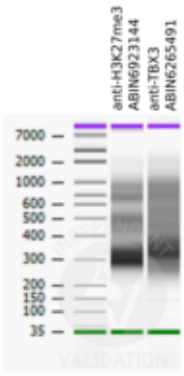
- Repeat twice for a total of three washes.
- Gently resuspend the ConA Beads in a volume of Binding Buffer corresponding to the original volume of bead slurry, i.e. 10 µL per sample.

- Cell immobilization – binding to Concanavalin A beads
  - Carefully vortex the cell suspension and add 10 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly and rotate for 10 min at RT.
- Cell permeabilization and primary antibody binding
  - Divide cell suspension into separate 2 mL microcentrifuge tubes, one for each antibody (500,000 cells per sample).
  - Place the microcentrifuge tubes on a magnetic stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 150 µL Digitonin Wash buffer (wash buffer with 0.025% (wt/vol) Digitonin) supplemented with 2 mM EDTA.
  - Gently vortex the microcentrifuge tubes until the beads are resuspended.
  - Add 1.5 µL antibody (Anti TBX3 (ABIN6265491), positive control, and negative control) to the respective tube, corresponding to a 1:100 dilution.
  - Rotate the microcentrifuge tubes ON at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 ml pipette tip.
  - Repeat once for a total of two washes.
- pA-MNase Binding
  - Place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Vortex the sample at low speed and add 150 µL pA-MNase solution at 700 ng/mL (1:200 dilution of a 140 µg/mL glycerol stock in Digitonin Wash Buffer) per sample, gently resuspending the beads by pipetting.
  - Rotate the microcentrifuge tubes for 1 h at 4 °C.
  - Spin down the liquid and place the tubes on a magnet stand until the fluid is clear. Remove the liquid carefully.
  - Remove the microcentrifuge tubes from the magnetic stand.
  - Resuspend with 1 mL Digitonin Wash Buffer and mix by inversion. If clumping occurs, gently remove the clumps with a 1 mL pipette tip.
  - Repeat once for a total of two washes.
- MNase digestion and release of pA-MNase-antibody-chromatin complexes
  - 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.
  - Place each tube at a low angle on the vortex mixer set to a low speed and add 100 µL

Digitonin Wash buffer per sample along the side of the tube.
- Place tubes in a heat block, kept on ice, and allow to chill.
- Add 2 µL 0.1 M CaCl2 to each sample.
- Incubate tubes at 0 °C for 30 min.
- Add 100 µL 2xSTOP buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.05% (wt/vol) Digitonin, 100 µg/mL RNAse A, 50 µg/mL Glycogen).
- Incubate tubes at 37 °C for 30 min.
- Place the tubes on a magnet stand until the fluid is clear.
- Transfer the supernatant containing the pA-MNase-bound digested chromatin fragments to fresh 1.5 mL microcentrifuge tubes.

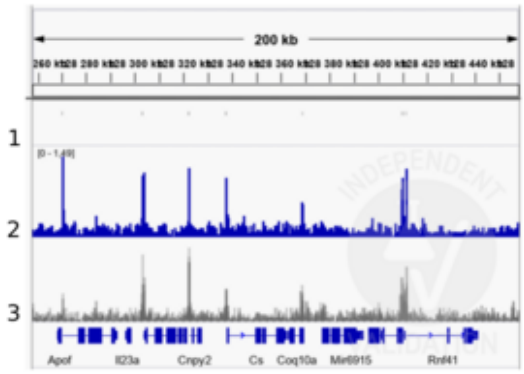
- DNA extraction
  - Add 2 µL 10% SDS to a final concentration of 0.1% and 2.5 µL Proteinase K (20 mg/mL) to each supernatant.
  - Gently vortex tubes at a low speed of approximately 1,100 rpm.
  - Incubate tubes at 50 °C for 1 h.
  - Add 200 µL PCI to tube.
  - Vortex tubes thoroughly at high speed until the liquid appears milky.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at RT for 5 min.
  - Carefully transfer to upper aqueous phase to a fresh 1.5 mL microcentrifuge tube containing 2 µL glycogen (diluted 1:10 to 2 mg/mL from the 20 mg/mL stock solution).
  - Add 20 µL 3 M NaOAc pH 5.2.
  - Add 400 µL 100% ethanol.
  - Place tubes for at -20 °C ON.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 5min.
  - Remove the liquid carefully with a pipette.
  - Wash pellet with 1ml 70% ethanol.
  - Centrifuge tubes in a tabletop centrifuge at 16,000 x g at 4 °C for 1 min.
  - Remove the liquid carefully with a pipette.
  - Air-dry the pellet, then dissolve in 30 µL 1 mM Tris-HCl, 0.1 mM EDTA.
- Library preparation and sequencing
  - Libraries were prepared using KAPA HyperPrep Kit using KAPA Dual-Indexed adapters according to protocol.
  - Samples were sequenced on an Illumina NextSeq 500 sequencer, using a NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 36bp PE.
- Peak calling
  - Reads were mapped to the GRCm38 (mm10) mouse genome using Bowtie2 with options: --local --very-sensitive- local --no-unal --no-mixed --no-discordant.
  - Peaks were called using MACS2 with options -f BAMPE --keep-dup all –nomodel.

Experimental Notes:	Peaks generated using ABIN6265491 for CUT&RUN aligned well with TBX3 ChIP-seq tracks from the same tissue (Zimmerli et. al., 2020).



Validation image no. 1 for anti-T-Box 3 (TBX3) antibody (ABIN6265491)

Bioanalyzer profiles comparing fragment size distributions between reads obtained from CUT&RUN using an anti-H3K27me3 CUT&RUN Positive Control antibody (ABIN6923144) and anti-TBX3 (ABIN6265491) after library preparation.



Validation image no. 2 for anti-T-Box 3 (TBX3) antibody (ABIN6265491)

Alignment tracks from CUT&RUN targeting TBX3 in mouse front limbs. 1. Peaks called by MACS2 from CUT&RUN data using anti-TBX3 antibody ABIN6265491 in cells derived from mouse embryonal front limbs. 2. Alignment track for CUT&RUN reads obtained using anti-TBX3 antibody ABIN6265491 in mouse embryonal front limbs. 2. Overlayed alignment tracks from ChIP-seq of TBX3 in mouse embryonal front limbs (Zimmerli et. al., 2020). Reads are normalized to sequencing depth per million reads.