Datasheet for ABIN5557532

# anti-STAT5A antibody

1 Validation

3 Images

Go to Product page

## Overview

| | |
|---|---|
| Quantity: | 100 µL |
| Target: | STAT5A |
| Reactivity: | Human, Mouse, Rat |
| Host: | Rabbit |
| Clonality: | Monoclonal |
| Conjugate: | This STAT5A antibody is un-conjugated |
| Application: | Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p)), Immunofluorescence (Cultured Cells) (IF (cc)) |

## Product Details

| | |
|---|---|
| Immunogen: | Recombinant human STAT5a protein, around C-terminal 100aa. |
| Clone: | 6E5 |
| Isotype: | IgG |
| Cross-Reactivity: | Human, Mouse, Rat |
| Purification: | Purified by Protein A. |

## Target Details

| | |
|---|---|
| Target: | STAT5A |
| Alternative Name: | STAT5a (STAT5A Products) |
| Background: | Synonyms: MGF, STAT5, Signal transducer and activator of transcription 5A, STAT5A |

## Target Details

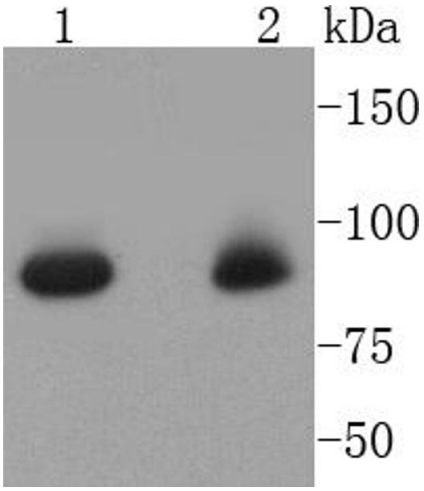
| | |
|---|---|
| | Background: Carries out a dual function: signal transduction and activation of transcription. Mediates cellular responses to the cytokine KITLG/SCF and other growth factors. Mediates cellular responses to ERBB4. May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the GAS element and activates PRL-induced transcription. Regulates the expression of milk proteins during lactation. |
| Gene ID: | 6776 |
| UniProt: | P42229 |
| Pathways: | JAK-STAT Signaling, RTK Signaling, Response to Growth Hormone Stimulus, C21-Steroid Hormone Metabolic Process, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, CXCR4-mediated Signaling Events, Activated T Cell Proliferation |

## Application Details

| | |
|---|---|
| Application Notes: | WB 1:300-5000<br>IHC-P 1:200-400<br>IF(ICC) 1:50-200 |
| Restrictions: | For Research Use only |

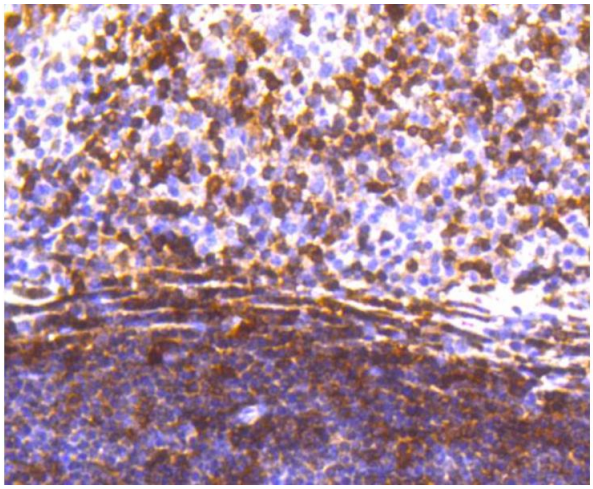
## Handling

| | |
|---|---|
| Format: | Liquid |
| Concentration: | 1 μg/μL |
| Buffer: | Aqueous buffered solution containing 1xTBS ( pH 7.4), 1 % BSA, 40 %Glycerol and 0.05 % Sodium Azide. |
| 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 |



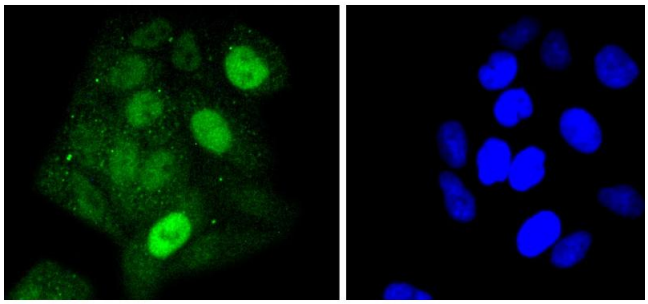

## Western Blotting

**Image 1.** Lane 1: Hela, Lane 2: K562 cell lysates, probed with STAT5a (6E5) Monoclonal Antibody at 1:1000 overnight at 4˚C. Followed by a conjugated secondary antibody.



## Immunohistochemistry (Paraffin-embedded Sections)

**Image 2.** Paraformaldehyde-fixed, paraffin embedded human tonsil section, Antigen retrieval by boiling in sodium citrate buffer (pH6) for 15min, Block endogenous peroxidase by 3% hydrogen peroxide for 30 minutes, Blocking buffer at 37°C for 20min, Antibody incubation with STAT5a (6E5) Monoclonal Antibody at 1:50 overnight at 4°C, followed by a conjugated secondary and DAB staining.



## Immunofluorescence (Cultured Cells)

**Image 3.** HeLa cells were stained with STAT5a (6E5) Monoclonal Antibody at [1:200] incubated overnight at 4C, followed by secondary antibody incubation, DAPI staining of the nuclei and detection.


**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: 104384

Date: Dec 07 2022

| | |
|---|---|
| Target: | STAT5A |
| Lot Number: | BA7271967 |
| 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. ABIN5557532 allows for CUT&RUN targeting of STAT5A in mouse forelimb tissue. |
| Primary Antibody: | ABIN5557532 |

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.

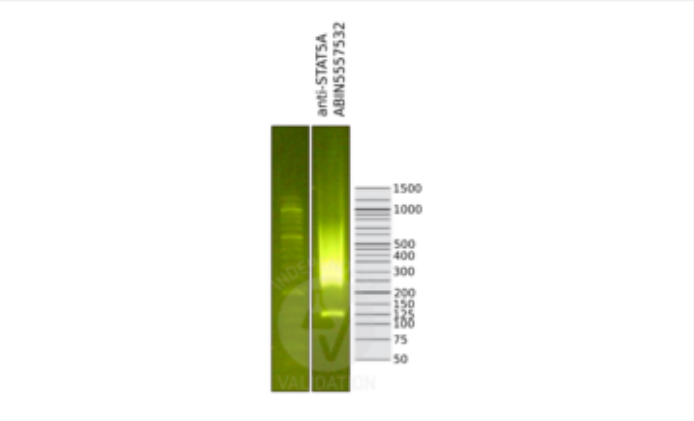
- Nuclei immobilization – binding to Concanavalin A beads
  - Carefully vortex the nuclei suspension and add 20 µL of the Con A beads in Binding Buffer to the cell suspension for each sample.
  - Close tube tightly incubates 10 min at 4 °C.
  - Put the 2 mL tube on the magnet stand and when the liquid 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-STAT5A antibody ABIN5557532, 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.

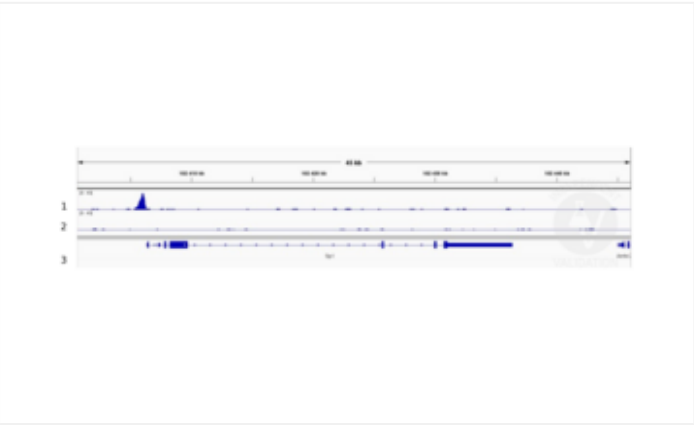
| | |
|---|---|
| Experimental Notes: | The protocol is published in Zambanini, G. et al. A New CUT&RUN Low Volume-Urea (LoV-U) protocol uncovers Wnt/β-catenin tissue-specific genomic targets. Development (2022). PMID 36355069 |

## Images for Validation report #104384




**Validation image no. 1 for anti-Signal Transducer and Activator of Transcription 5A (STAT5A) antibody (ABIN5557532)**

Library profiles comparing fragment size distributions on an E-Gel EX 2% agarose gel (Thermo Fisher). Fragments obtained from CUT&RUN using STAT5A ABIN5557532 (right) after library preparation, compared to the E-Gel Sizing DNA Ladder (Thermo Fisher) (left).



**Validation image no. 2 for anti-Signal Transducer and Activator of Transcription 5A (STAT5A) antibody (ABIN5557532)**

1. Alignment tracks from CUT&RUN targeting STAT5A in mouse fore limb (11.5) cells using ABIN5557532, showing the Sp1 locus. 2. Alignment tracks using negative control IgG, ABIN1019613. 3. RefSeq Genes.