abcam

## ab5832

# Anti-hnRNP A1 antibody [9H10]

Anti-hnRNP A1 antibody [9H10] (ab5832) is a mouse monoclonal antibody detecting hnRNP A1 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF, ELISA**. Suitable for **Human, Mouse**.

- Over 50 publications
- Trusted since 2003

## Key facts

| | |
|---|---|
| **Isotype** | IgG2b |
| **Host species** | Mouse |
| **Storage buffer** | Preservative: 0.1% Sodium azide Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | Native Full Length Protein corresponding to Human HNRNPA1. Database link P09651 |
| **Clone number** | 9H10 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 1 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |
| **Purification notes** | Purified from tissue culture supernatant. |

## Reactivity data

### IHC-P

**Tested**

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1 µg/mL |
| **Notes** | Perform heat-mediated antigen retrieval before commencing with IHC staining protocol. |

### Predicted

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | This antibody does not IP the hnRNP complex. |

### Predicted

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1 µg for $10^6$ Cells |
| **Notes** | ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody. |

### Predicted

| | |
|---|---|
| **Species** | Mouse |

| | |
|---|---|
| **Dilution info** | - |
| **Notes** | - |

## ELISA

### Expected

| | |
|---|---|
| **Species** | Mouse, Human |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | See Abreview (April 2, 2007). |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | See Abreview (April 2, 2007). |

## Target data

See full target information HNRNPA1

| | |
|---|---|
| **Function** | Involved in the packaging of pre-mRNA into hnRNP particles, transport of poly(A) mRNA from the nucleus to the cytoplasm and modulation of splice site selection (PubMed:17371836). Plays a role in the splicing of pyruvate kinase PKM by binding repressively to sequences flanking PKM exon 9, inhibiting exon 9 inclusion and resulting in exon 10 inclusion and production of the PKM M2 isoform (PubMed:20010808). Binds to the IRES and thereby inhibits the translation of the apoptosis protease activating factor APAF1 (PubMed:31498791). May bind to specific miRNA hairpins (PubMed:28431233). (Microbial infection) May play a role in HCV RNA replication. (Microbial infection) Cleavage by Enterovirus 71 protease 3C results in increased translation of apoptosis protease activating factor APAF1, leading to apoptosis. |

## Storage

| | |
|---|---|
| **Shipped at conditions** | Blue Ice |
| **Appropriate short-term storage duration** | 1-2 weeks |
| **Appropriate short-term storage conditions** | +4°C |
| **Appropriate long-term storage conditions** | -20°C |
| **Aliquoting information** | Upon delivery aliquot |
| **Storage information** | Avoid freeze / thaw cycle |

## Notes

**What is this antibody validated in?**
Anti-hnRNP A1 antibody [9H10] (ab5832) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), ELISA in Human, Mouse samples.

**What is the molecular weight of hnRNP A1?**
Anti-hnRNP A1 [9H10] (ab5832) specifically detects a band for hnRNP A1 (UniProt: P09651) at a molecular weight of 38kDa.

**Trusted by the scientific community**
Anti-hnRNP A1 [9H10] (ab5832) was first used in a scientific publication in 2003 and has been cited over 50 times in peer-reviewed journals.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) also known just as hnRNP A1 is a multifunctional protein that plays important roles in RNA processing mechanisms. It is a member of the hnRNP protein family involved in the packaging of nascent pre-mRNA. hnRNP A1 has a molecular mass of approximately 34-38 kDa and shows a widespread expression in various cells and tissues. This protein is recognized for its ability to bind to RNA molecules contributing to their proper processing splicing and transport within the cellular context. |
| **Biological function summary** | HnRNP A1 is involved in RNA binding and splicing contributing to the formation of spliceosomes the complexes responsible for pre-mRNA splicing. The protein aids in alternative splicing influencing mRNA diversity and stability. hnRNP A1 interacts with other proteins in the hnRNP family remodeling ribonucleoprotein complexes and affecting their functions. These interactions ensure proper mRNA maturation impacting gene expression regulation within the cell. |
| **Pathways** | HnRNP A1 is integral to critical cellular mechanisms such as the regulation of mRNA transport and splicing. It influences the DNA damage response pathway by modulating gene expression required for cellular repair and stability. hnRNP A1 also cooperates with other RNA binding proteins like the splicing factors SR proteins to orchestrate the splicing machinery. These interactions are pivotal in maintaining cellular homeostasis and response to stress. |
| **Associated diseases and disorders** | HnRNP A1 is notably related to neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS) and certain types of cancer. Abnormalities in its expression or mutations can lead to disrupted cellular processes contributing to disease pathology. In ALS dysregulation of hnRNP A1 affects neuronal RNA metabolism and is associated with the TDP-43 protein a marker of neurodegeneration. In cancer hnRNP A1 influences tumor development through its role in alternative splicing and gene expression modification linking it to oncogenic signaling pathways. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

We do not recommend this combination. It is not covered by our product promise.

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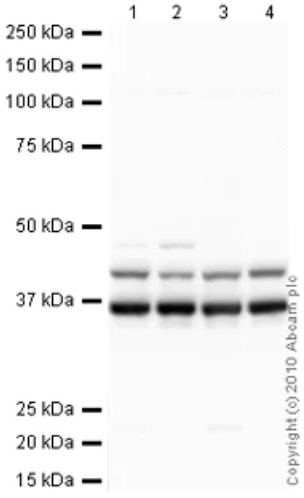
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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## 9 product images




### Western blot - Anti-hnRNP A1 antibody [9H10] (ab5832)

HnRNP A1 Western blot staining using mouse Anti-hnRNP A1 antibody

All lanes:
Western blot - Anti-hnRNP A1 antibody [9H10] (ab5832) at 1 µg/mL

Lane 1:
HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

Lane 2:
Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate at 10 µg

Lane 3:
HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate at 10 µg

Lane 4:
HEK293 (Human embryonic kidney cell line) Whole Cell Lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Developed using the ECL technique.

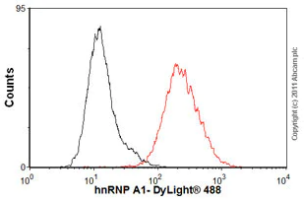
Performed under reducing conditions.

Predicted band size: 38 kDa

Observed band size: 37 kDa, 43 kDa

Exposure time: 30s

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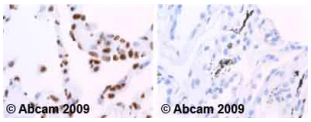



### Flow Cytometry - Anti-hnRNP A1 antibody [9H10] (ab5832)

HnRNP A1 Flow Cytometry staining using mouse Anti-hnRNP A1 antibody

Overlay histogram showing Jurkat cells stained with ab5832 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-

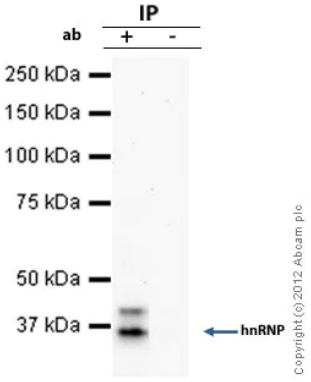
protein interactions followed by the antibody (ab5832, 1µg/1x10$^6$ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10$^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with 4% paraformaldehyde/permeabilized in 0.1% PBS-Tween used under the same conditions.




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-hnRNP A1 antibody [9H10] (ab5832)

HnRNP A1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-hnRNP A1 antibody

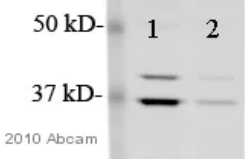
ab5832 staining human lung. Staining is localized to the nucleus.
Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.
Sections were stained using an automated system (Dako PT Link), at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 antigen retrieval buffer, citrate pH 6.0. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.




## Immunoprecipitation - Anti-hnRNP A1 antibody [9H10] (ab5832)

hnRNP A1 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Mouse monoclonal to hnRNP A1 (ab5832) and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70ºC; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab5832.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: 37kDa: hnRNP A1; 42kDa:We are unsure as to the identity of this extra band.
All lanes:
Immunoprecipitation - Anti-hnRNP A1 antibody [9H10] (ab5832)

Predicted band size: 38 kDa




This image is courtesy of an anonymous customer review.

## Western blot - Anti-hnRNP A1 antibody [9H10] (ab5832)

Knockdown for 72 hours.
All lanes:
Western blot - Anti-hnRNP A1 antibody [9H10] (ab5832) at 1/1000 dilution

Lane 1:
Mouse NSC34 whole cell lysate at 10 µg with control siRNA

Lane 2:

Mouse NSC34 whole cell lysate at 10 µg with hnRNP A1 siRNA

Secondary

All lanes:
IRDye® 700DX-conjugated Donkey anti-Mouse IgG at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 38 kDa

Exposure time: 1min

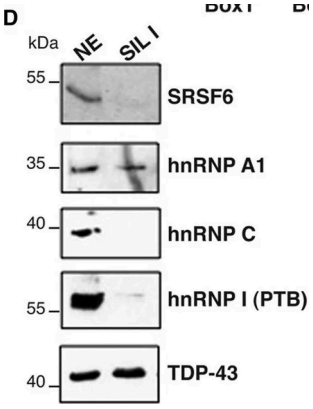



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## Western blot - Anti-hnRNP A1 antibody [9H10] (ab5832)

HnRNP A1 Western blot staining using mouse Anti-hnRNP A1 antibody

Western Blotting using Anti-hnRNP A1 antibody [9H10], ab5832. Publication image from Buratti, E. et al., 2013, Nucleic Acids Res, 23863836. Legend direct from paper.

The first 44 nt of the Ron ESS element mediate splicing inhibition activity and interact with hnRNP A1. (A) Sequence of the previously identified 84 nt ESS. The position of SIL-I, SIL-II and SIL-III elements is indicated. Numbers refer to the cDNA sequence. (B) The entire ESS, SIL-I, SIL-II, SIL-III and ESE elements were cloned into the second exon of the human β-globin (Hβ) minigene. Minigenes were transfected into KATOIII cells; 24 h later RNAs were analyzed by RT-PCR with primer set β-globin_A and D (8) to detect both spliced (mRNA) and unspliced transcripts (pre-mRNA). Total (pre-mRNA plus mRNA, primers β-globin_A and Hbeta/CONTROL) and Spliced (mRNA, primers β-globin_A and Hbeta/DWsplicing) molecules were quantified by using qRT-PCR analysis; the ratio between Spliced and Total was plotted in the histogram by using the Hβ vector as a reference value. (C) Putative binding sites for hnRNP A1 (Box1) and SRSF6 (Box2) identified within SIL-I element by bioinformatics analysis. (D) In vitro pull-down assay with HeLa nuclear extracts (NE) and with a SIL-I riboprobe containing at its 3′ end the binding site for splicing factor TDP-43, as an internal control of pull-down efficiency. Materials were analyzed by western blotting with antibodies for indicated proteins.

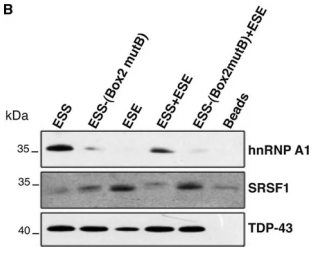



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HnRNP A1 binding to the Ron ESS interferes with SRSF1 binding to the ESE element. (A) The panel on the left shows the sequence of the wild type SIL-I probe and its different mutated versions. Right panel: KATOIII cells were transfected with the indicated Hβ vectors and RNAs were analyzed by RT-PCR as in Figure 1B. (B) In vitro pull-down assay with HeLa nuclear extracts and the indicated RNA probes: WT Ron ESS (84 nt), ESS mutated in Box2 (mutB = TGCGGC replaced with ATCGAC), the Ron ESE (67 nt), ESE plus ESS elements (151 nt) WT or mutated in Box2. Affinity bound proteins were analyzed by western blotting with the indicated antibodies. (C) RNA immunoprecipitation (IP) following UV cross-linking using anti-hnRNP A1 mAb 9H10 or IgG on HeLa cells. The abundance of Ron exon 12 or, as control, 7SL, 5S and tRNA-Leu enrichments were measured by qRT-PCR.

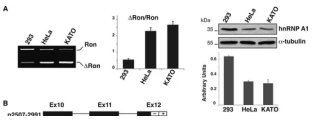


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HnRNP A1 Western blot staining using mouse Anti-hnRNP A1 antibody

Western Blotting using Anti-hnRNP A1 antibody [9H10], ab5832. Publication image from Buratti, E. et al., 2013, Nucleic Acids Res, 23863836. Legend direct from paper.

HnRNP A1 inhibits the production of the δRon transcript. (A) RT-PCR analysis of the splicing profile of endogenous Ron transcripts in HEK-293, HeLa and KATOIII cells with primers 2507 and 2991 (8). The histogram shows the qRT-PCR analysis of the total hnRNP A1 transcripts (primers hnRNPA1for and rev). On the right, western blot analysis of hnRNP A1 andα-tubulin protein levels in the indicated cell lines. (B) Scheme of the Ron minigene p2507–2991 (8) and of a derivative mutated in the ESS sequence (pMut-SIL-I). (C) HEK-293 and (D) KATOIII cells were transfected with the indicated minigenes and RNAs analyzed with primers 2507 and BGHrev (8); t-Ron and t-δRon indicate minigene transcripts generated by inclusion and skipping of exon 11, while histograms show the t-δRon/t-Ron measured in three independent experiments. (E) HeLa cells were transfected with GFP-hnRNP A1 or with the empty vector ( ‘Vector’ ). The expression of the overexpressed protein was verified by western blotting with anti-GFP and anti-α-tubulin antibodies. On the right, p2507–2991 and pMut-SIL-I minigenes were co-transfected into HeLa cells with the GFP-tagged hnRNP A1 or the empty vector. Total RNAs were analyzed by RT-PCR as in Figure 3B.

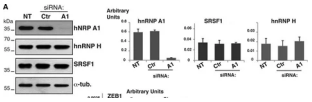


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HnRNP A1 Western blot staining using mouse Anti-hnRNP A1 antibody

Western Blotting using Anti-hnRNP A1 antibody [9H10], ab5832. Publication image from Buratti, E. et al., 2013, Nucleic Acids Res, 23863836. Legend direct from paper.

HnRNP A1 affects the MET program. (A) HEK-293 cells not treated (NT) or transfected with hnRNP A1 (A1) or with control siRNAs (Ctr). Total cell extracts were probed by western blotting with the following antibodies: anti hnRNP A1 (9H10), anti-hnRNP H, anti-SRSF1 (mAb96) and anti-α-tubulin; the three histograms on the right show the relative level of hnRNP A1, SRSF1 and hnRNP H transcripts quantified by qRT-PCR. (B) The same cells (not treated or transfected with siRNA oligos) were analyzed in (i) RT-PCR to determine the splicing profile of the endogenous Ron transcripts as in Figure 3A and (ii) qRT-PCR for the expression level of the indicated EMT markers. Cells morphologies were examined under a phase-contrast microscope (objective 10x with additional magnification 1.6x). (C) HeLa cells were transfected with T7-hnRNP A1 or with the empty vector ( ‘Vector’ ) with an efficiency of ☒60%; RNAs were analyzed with RT-PCR (as in Figure 3A) to determine the splicing profile of Ron exon 11 and cell extracts were analyzed in western blotting to assess the abundance of the indicated proteins and the expression of T7-hnRNP A1. The same cells were also analyzed in qRT-PCR for the expression levels of selected EMT markers. Panel on the right shows the results of the wound healing assay. Transient transfected HeLa cells were grown to confluence and wounded by dragging a 200 µl pipette tip through the monolayer (0 h); cell migration was measured at designated times (5, 10 and 24 h) after wounding. (D) MDA-MB-435S cells stably transfected with T7-SRSF1 (Cl.SF2) or with the empty vector (Cl.Vector) (29). Cl.SF2 and Cl.Vector cells were transfected with T7-hnRNP A1 or with the empty vector ( ‘Vector’ ) as indicated; total cell extracts were analyzed in western blotting to assess the level of the indicated proteins. (E) Total RNAs from the same cells were analyzed by RT-PCR (as in Figure 3A); the histogram below shows the δRon/Ron ratio. Expression level of the epithelial marker E-cadherin in the different cells as assessed by qRT-PCR is also shown.

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