abcam

ab251486

Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20175
Purification technique	Affinity purification Protein A
Specificity	Based on internal testing in Dot blot, this antibody cross reacts with hnRNP A0 (citrulline R81/85) and hnRNP A2B1 (citrulline R95/99). The immunogen used for this product shares 100% homology with hnRNP A1-like 2. Cross-reactivity with this protein has not been confirmed experimentally. Treatment is required for detection of the target protein. See Protocol section for detailed information. Protocol .
Concentration	1.087 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide, Recombinant fragment - Human
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Mouse, Rat

Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant fragment - Human, Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide, Recombinant fragment - Human
Dilution info	-
Notes	-

Target data

See full target information HNRNPA1 citR88 + R92

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 conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab251486 is the carrier-free version of ab208027.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Treatment Conditions:

Chemical treatment is required for detection of endogenous citrullinated proteins in cell lines. PADs (including PADI2 and PADI4) are the enzymes that hydrolyze arginine to citrulline, in the presence of Ca^{2+} and ionomycin. Please refer to PMID: 26360112

Controls:
- Mock (no transfection)
- Transfection reagent–only control
- Empty vector control
- Vehicle control for chemical treatment

Generation of PADI2 or PADI4 overexpression cell line and treatment with $CaCl_2$/Ionomycin:

1. Prior to transfection with PADI2 or PADI4 expression vector, seed cells in complete medium and place in a 37°C, 5% CO_2 incubator for 24 hours or until cells reach 70-80% confluence.

For a single T-175 flask we recommend seeding the following common cell lines as follows in 30 mL media:
- HEK-293T: $1x10^7$ cells
- MCF7: $2.5\text{-}4x10^6$ cells

2. Once cells have reached confluence, prepare the PADI2 or PADI4 expression vector for transfection using your preferred method (PMID: 33976969).
3. 24-48 hours after transfection, remove the medium from the cells and replace with fresh complete medium containing 10 mM $CaCl_2$ (ab285384) and 10 uM Ionomycin (ab120116) and incubate for 2-4 hours.

Key Considerations:
For alternative cell lines, you should confirm the transfectability, growth rate, and sensitivity of the cell line to the planned chemical treatment. Incubation times and treatment length may need to be adjusted.

Western Blot Conditions:
Recommended SDS-PAGE Gel Type: 4-20% Tris-glycine
Recommended Running Buffer: Tris-Glycine-SDS
Primary: 1/5000 dilution at 4°C overnight or room temperature for 1 hour
Blocking: 5% NFDM at room temperature for 1 hour

Positive Control: Cell line overexpressing PADI4 and treated with $CaCl_2$/Ionomycin
Negative Control: Cell line overexpressing PADI4

Additional resources:
Lysate preparation protocol
Lysate preparation protocol - nuclear proteins
Lysate preparation protocol - low molecular weight proteins
Western blot troubleshooting

Flow Cytometry Conditions:
Primary: 1/5000 dilution at 4°C for 30 minutes
Fixation: 4% PFA at room temperature for 10 minutes
Permeabilization:

Positive Control: Cell line overexpressing PADI4 and treated with CaCl2/Ionomycin
Negative Control: Cell line overexpressing PADI4
Secondary only control:

Additional resources:
Flow Cytometry protocol
Flow cytometry troubleshooting

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

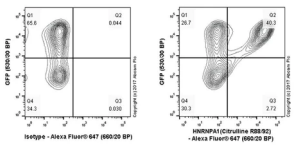
We do not recommend this combination. It is not covered by our product promise.

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.

4 product images

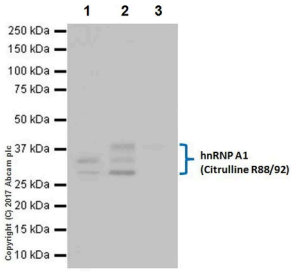


Flow Cytometry (Intracellular) - Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] - BSA and Azide free (ab251486)

HnRNP A1 (citrulline R88 + R92) Flow Cytometry (Intracellular) staining using rabbit Anti-hnRNP A1 (citrulline R88 + R92) antibody

This data was developed using ab208027, the same antibody clone in a different buffer formulation.
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with PADI4 expression vector, then treated with 10 mM CaCl2 and 10 µM Ionomycin for 2 hours, labeling hnRNP A1 with ab208027 at 1/5000 dilution (right panel) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150079) at 1/2000 dilution was used as the secondary antibody.

The CaCl2 and Ionomycin treated GFP positive cell population gives a positive signal. (Right panel, Q2).



Immunoprecipitation - Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] - BSA and Azide free (ab251486)

This data was developed using ab208027, the same antibody clone in a different buffer formulation.
hnRNP A1 was immunoprecipitated from 0.35 mg HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) with ab208027 at 1/30 dilution, whole cell lysate. Western blot was performed from the immunoprecipitate using ab208027 at 1/10000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: HEK-293T transfected with PADI4 expression vector, then treated with 10 mM $CaCl_2$ and 10µM Ionomycin for 2 hours whole cell lysate 10 µg (Input).

Lane 2: ab208027 IP in HEK-293T transfected with PADI4 expression vector, then treated with 10 mM $CaCl_2$ and 10 µM Ionomycin for 2 hours whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab208027 in HEK-293T transfected with PADI4 expression vector, then treated with 10 mM $CaCl_2$ and 10 µM Ionomycin for 2 hours whole cell lysate.

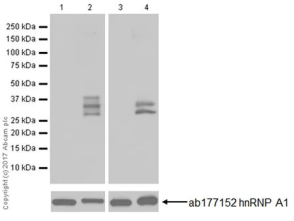
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 1 second.

All lanes:
Immunoprecipitation - Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] (ab208027)

Predicted band size: 38 kDa

Observed band size: 31 kDa, 34 kDa, 37 kDa



Western blot - Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] - BSA and Azide free (ab251486)

This data was developed using ab208027, the same antibody clone in a different buffer formulation.

hnRNP A1 (Uniprot P09651), isoform A1-A (34kD) is twenty times more abundant than isoform A1-B (39kD). The molecular weights of hnRNP A1L2 (Q32P51), hnRNP A0 (Q13151) and hnRNP A2B1 (P22626) are 34kD, 31kD and 37kD respectively.

PAD enzymes (including PADI2 and PADI4) hydrolyze arginine to form citrulline, Ca^{2+} and ionomycin are also required for this catalytic reaction (PMID: 26360112).

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times: Lanes 1-2: 1 second; Lanes 3-4: 2 seconds.
All lanes:
Western blot - Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] (ab208027) at 1/5000 dilution

Lane 1:
HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with empty vector (control) then treated with 10 mM CaCl2 and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

Lane 2:
HEK-293T transfected with a PADI4 expression vector then treated with 10 mM CaCl2 and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

Lane 3:
HEK-293T transfected with an empty vector (control) then treated with 10 mM CaCl2 and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

Lane 4:
HEK-293T transfected with a PADI2 expression vector then treated with 10 mM CaCl2 and 10 µM Ionomycin for 2 hours, whole cell lysate at 20 µg

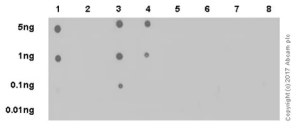
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 38 kDa

Observed band size: 31 kDa, 37 kDa, 39 kDa



Dot Blot - Anti-hnRNP A1 (citrulline R88 + R92) antibody [EPR20175] - BSA and Azide free (ab251486)

HnRNP A1 (citrulline R88 + R92) Dot Blot staining using rabbit Anti-hnRNP A1 (citrulline R88 + R92) antibody

This data was developed using ab208027, the same antibody clone in a different buffer formulation.
Dot Blot analysis of hnRNP A1 (citrulline R88 + R92) labeled with ab208027 at 1/1000 dilution.

Lane 1: hnRNP A1 (citrulline R88/92) peptide.
Lane 2: hnRNP A3 (citrulline R109/113) peptide.
Lane 3: hnRNP A0 (citrulline R81/85) peptide.
Lane 4: hnRNP A2B1 (citrulline R95/99) peptide.
Lane 5: CCDC51 (citrulline R142) peptide.
Lane 6: hnRNP A1 non-citrulline peptide.
Lane 7: hnRNP A1 (citrulline R88) peptide.
Lane 8: hnRNP A1 (citrulline R92) peptide.

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.