abcam

Product datasheet

Anti-HNRPAB antibody [EPR16944] ab199724





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Overview

Product name Anti-HNRPAB antibody [EPR16944]

Description Rabbit monoclonal [EPR16944] to HNRPAB

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), IP, IHC-P, WB, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HEK293T, K562, Jurkat, HeLa, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates;

> Human fetal brain lysate; Mouse brain and rat brain lysates. IHC-P: Human cervix carcinoma, mouse liver and rat cardiac muscle tissues. ICC/IF: Jurkat cells. IP: K562 whole cell lysate.

General notes 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 production

For more information see here.

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

Properties

Liquid **Form**

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal Clone number EPR16944

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab199724 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use a concentration of 1 µg/ml.
IP		1/30.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 42 kDa (predicted molecular weight: 36 kDa).
ICC/IF		1/500.

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Function Binds single-stranded RNA. Has a high affinity for G-rich and U-rich regions of hnRNA. Also binds

to APOB mRNA transcripts around the RNA editing site.

Tissue specificity Ubiquitous.

Sequence similarities Contains 2 RRM (RNA recognition motif) domains.

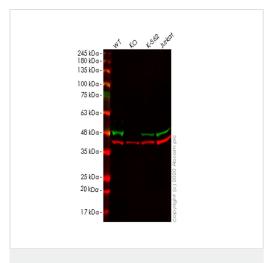
Post-translational

modifications

Dimethylation at Arg-322 is probably asymmetric.

Cellular localizationNucleus. Cytoplasm. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Images



Western blot - Anti-HNRPAB antibody [EPR16944] (ab199724)

All lanes : Anti-HNRPAB antibody [EPR16944] (ab199724) at 1/1000 dilution

Lane 1: Wild-type HEK293T cell lysate

Lane 2: HNRNPAB knockout HEK293T cell lysate

Lane 3 : K-562 cell lysate

Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

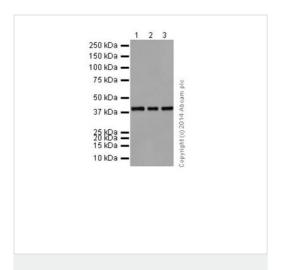
Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 36 kDa **Observed band size:** 48 kDa

Lanes 1-4: Merged signal (red and green). Green - ab199724 observed at 48 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab199724 Anti-HNRPAB antibody [EPR16944] was shown to specifically react with HNRPAB in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266459 (knockout cell lysate ab266459 (knockout cell lysate ab266459 (knockout cell lysate ab266459 (knockout samples were subjected to SDS-PAGE. ab199724 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216773) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-HNRPAB antibody [EPR16944] (ab199724)

All lanes : Anti-HNRPAB antibody [EPR16944] (ab199724) at 1/5000 dilution

Lane 1 : K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate

Lane 2 : Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate

Lane 3: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

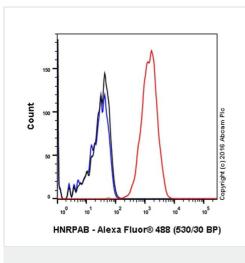
All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 36 kDa **Observed band size:** 42 kDa

Exposure time: 5 seconds

The expression profile observed is consistent with what has been described in the literature PMID: 22332140.

Blocking/Dilution buffer: 5% NFDM/TBST.

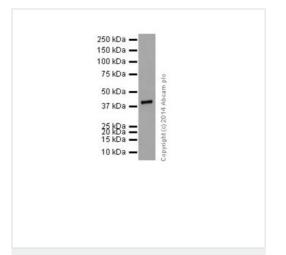


Flow Cytometry (Intracellular) - Anti-HNRPAB antibody [EPR16944] (ab199724)

ab199724 staining HNRPAB in Jurkat (human acute T cell leukemia) cellsby intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/2700. A goat anti rabbit IgG (Alexa Fluor[®] 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-HNRPAB antibody [EPR16944] (ab199724)

Anti-HNRPAB antibody [EPR16944] (ab199724) at 1/5000 dilution + Human fetal brain lysate at 20 µg

Secondary

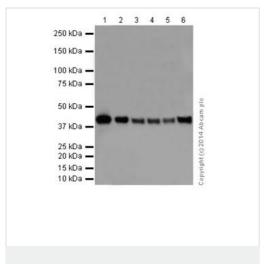
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 36 kDa **Observed band size:** 42 kDa

Exposure time: 10 seconds

The expression profile observed is consistent with what has been described in the literature PMID: 22332140.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-HNRPAB antibody [EPR16944] (ab199724)

All lanes : Anti-HNRPAB antibody [EPR16944] (ab199724) at 1/1000 dilution

Lane 1: Mouse brain lysates

Lane 2: Rat brain lysates

Lane 3: C6 (Rat glial tumor cells) whole cell lysate

Lane 4: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysate

Lane 5: PC-12 (Rat adrenal gland pheochromocytoma) whole cell

lysate

Lane 6: NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

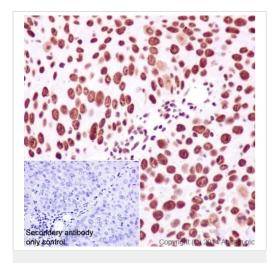
All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 36 kDa **Observed band size:** 42 kDa

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The expression profile observed is consistent with what has been described in the literature PMID: 22332140.

Blocking/Dilution buffer: 5% NFDM/TBST.

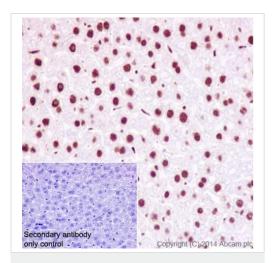


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HNRPAB antibody
[EPR16944] (ab199724)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling HNRPAB with ab199724 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on Human cervix carcinoma tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

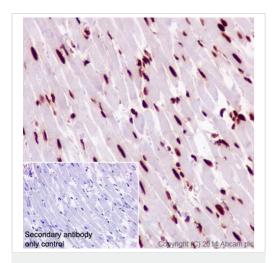


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HNRPAB antibody
[EPR16944] (ab199724)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling HNRPAB with ab199724 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on mouse liver tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

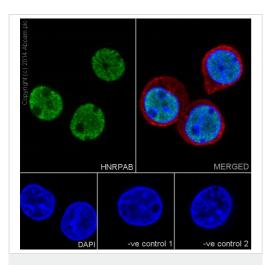


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HNRPAB antibody
[EPR16944] (ab199724)

Immunohistochemical analysis of paraffin-embedded rat cardiac muscle tissue labeling HNRPAB with ab199724 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear staining on rat cardiac muscle tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

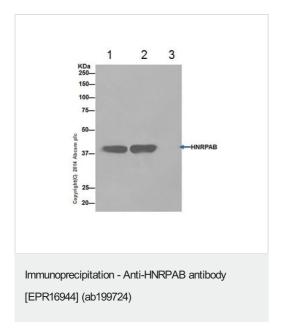


Immunocytochemistry/ Immunofluorescence - Anti-HNRPAB antibody [EPR16944] (ab199724)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling HNRPAB with ab199724 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Nuclear staining on Jurkat cell line is observed. The nuclear counterstain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:-

-ve control 1: ab199724 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.



HNRPAB was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cells from bone marrow) whole cell lysate with ab199724 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab199724 at 1/1000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: K562 whole cell lysate 10 µg (Input). Lane 2: ab199724 IP in K562 whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab199724 in K562 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



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