abcam

Product datasheet

Anti-Aly/Ref antibody [EPR17942] ab202894

Recombinant RabMAb

9 References 11 Images

Overview

Product name Anti-Aly/Ref antibody [EPR17942]

Description Rabbit monoclonal [EPR17942] to Aly/Ref

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: PC-3, HeLa, HepG2, HEK293 whole cell lysates; Human fetal brain, fetal heart and fetal

kidney lysates; Mouse brain and heart lysates; rat brain and heart lysates. IHC-P: Human cervix carcinoma, Mouse cerebral cortex and rat liver tissues. ICC/IF: HeLa and C6 cells. Flow Cyt:

HeLa cells. IP: HepG2 whole cell lysate.

General notesThis product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

Improved sensitivity and specificityLong-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

Form Liquid

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

ClonalityMonoclonalClone numberEPR17942

1

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab202894 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)		1/150.
WB		1/2000. Detects a band of approximately 27 kDa (predicted molecular weight: 27 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/250.
IP		1/40.

Target

Function

Component of the THO subcomplex of the TREX complex. The TREX complex specifically associates with spliced mRNA and not with unspliced pre-mRNA. It is recruited to spliced mRNAs by a transcription-independent mechanism. Binds to mRNA upstream of the exon-junction complex (EJC) and is recruited in a splicing- and cap-dependent manner to a region near the 5' end of the mRNA where it functions in mRNA export. The recruitment occurs via an interaction between THOC4 and the cap-binding protein NCBP1. DDX39B functions as a bridge between THOC4 and the THO complex. TREX complex is essential for the export of Kaposi's sarcoma-associated herpesvirus (KSHV) intronless mRNAs and infectious virus production. The recruitment of the TREX complex to the intronless viral mRNA occurs via an interaction between KSHV ORF57 protein and THOC4. THOC4 in conjunction with THOC5 functions in NXF1-NXT1 mediated nuclear export of HSP70 mRNA.

Component of a splicing-dependent multiprotein exon junction complex (EJC) deposited at splice junction on mRNAs. The EJC is a dynamic structure consisting of a few core proteins and several more peripheral nuclear and cytoplasmic associated factors that join the complex only transiently either during EJC assembly or during subsequent mRNA metabolism. Acts as chaperone and promotes the dimerization of transcription factors containing basic leucine zipper (bZIP) domains and thereby promotes transcriptional activation. Plays a role in mRNA processing and export. May function as scaffold that mediates interactions between proteins and/or RNA. Is part of the exon junction complex that remains associated with spliced mRNA and plays an important role in mRNA export and nonsense-mediated RNA decay. Directs mRNA derived from Herpes simplex virus intron-less genes to the NXF1-mediated export pathway.

Sequence similarities

Belongs to the THOC4 family.

Contains 1 RRM (RNA recognition motif) domain.

Post-translational modifications

Arg-50 and Arg-204 are dimethylated, probably to asymmetric dimethylarginine.

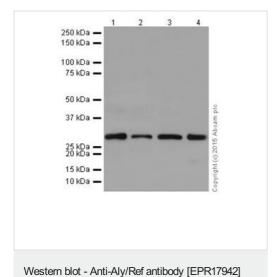
Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization

Nucleus. Nucleus speckle. Cytoplasm. Colocalizes with the core EJC, THOC4, NXF1 and DDX39B in the nucleus and nuclear speckles. Travels to the cytoplasm as part of the exon junction complex (EJC) bound to mRNA.

Images

(ab202894)



All lanes : Anti-Aly/Ref antibody [EPR17942] (ab202894) at 1/10000 dilution

Lane 1 : PC-3 (Human prostate cancer cell line) whole cell lysate

Lane 2 : HepG2 (Human liver hepatocellular carcinoma) whole cell

lysate

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

Lane 4: HEK293 (Human epithelial cells from embryonic kidney) whole cell lysate

Lysates/proteins at 20 µg per lane.

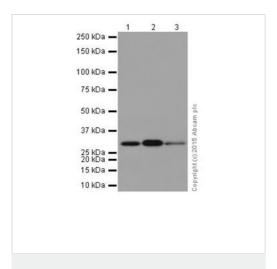
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Predicted band size: 27 kDa **Observed band size:** 27 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Aly/Ref antibody [EPR17942] (ab202894)

All lanes: Anti-Aly/Ref antibody [EPR17942] (ab202894) at 1/2000 dilution

Lane 1 : Human fetal brain lysate

Lane 2 : Human fetal heart lysate

Lane 3 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/1000 dilution

Predicted band size: 27 kDa **Observed band size:** 27 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 3 4

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-Aly/Ref antibody [EPR17942] (ab202894)

All lanes : Anti-Aly/Ref antibody [EPR17942] (ab202894) at 1/2000 dilution

Lane 1 : Mouse brain lysate
Lane 2 : Mouse heart lysate
Lane 3 : Rat brain lysate
Lane 4 : Rat heart lysate

Lysates/proteins at 10 µg per lane.

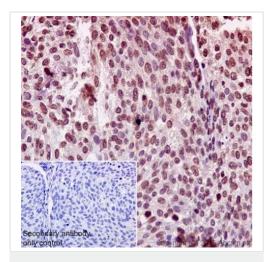
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Predicted band size: 27 kDa **Observed band size:** 27 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

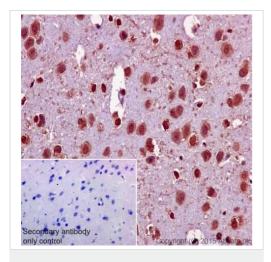


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Aly/Ref antibody
[EPR17942] (ab202894)

Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling Aly/Ref with ab202894 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and weakly cytoplasmic 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) at 1/500 dilution.

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

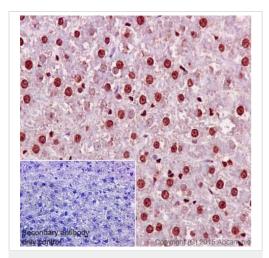


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Aly/Ref antibody
[EPR17942] (ab202894)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling Aly/Ref with ab202894 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and weakly cytoplasmic staining on mouse cerebral cortex 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) at 1/500 dilution.

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

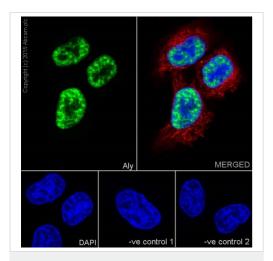


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Aly/Ref antibody
[EPR17942] (ab202894)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling Aly/Ref with ab202894 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nuclear and weakly cytoplasmic staining on rat liver 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) at 1/500 dilution.

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

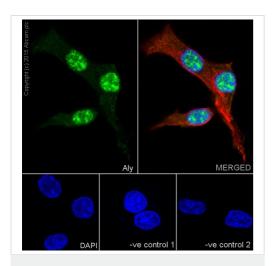


Immunocytochemistry/ Immunofluorescence - Anti-Aly/Ref antibody [EPR17942] (ab202894)

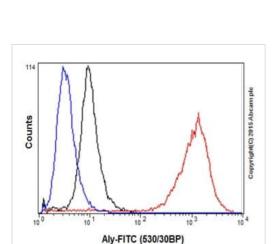
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Aly/Ref with ab202894 at 1/250 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain 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 1/1000 dilution (red).

The negative controls are as follows:

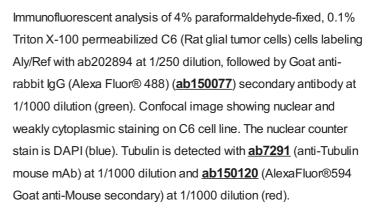
-ve control 1: ab202894 at 1/250 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Aly/Ref antibody [EPR17942] (ab202894)



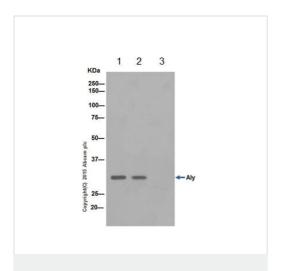
Flow Cytometry (Intracellular) - Anti-Aly/Ref antibody [EPR17942] (ab202894)



The negative controls are as follows:

-ve control 1: ab202894 at 1/250 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 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 1/1000 dilution.

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Aly/Ref with ab202894 at 1/150 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Aly/Ref antibody [EPR17942] (ab202894)

Aly/Ref was immunoprecipitated from 1mg of HepG2 (Human liver hepatocellular carcinoma) whole cell lysate with ab202894 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab202894 at 1/5000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: HepG2 whole cell lysate 10 µg (Input). Lane 2: ab202894 IP in HepG2 whole cell lysate. Lane 3: Rabbit monoclonal lgG (ab172730) instead of ab202894 in HepG2 whole cell lysate.

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

Exposure time: 8 seconds.



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