abcam

Product datasheet

Anti-RPA70 antibody [EPR3472] ab79398



★★★★★ 6 Abreviews 65 References 11 Images

Overview

Product name Anti-RPA70 antibody [EPR3472]

Description Rabbit monoclonal [EPR3472] to RPA70

Host species Rabbit

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

Species reactivity Reacts with: Human

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

Positive control WB: A549, HEK-293 and HeLa whole cell lysate (ab150035) IHC-P: Human cervical squamous

cell carcinoma tissue. ICC/IF: A549 cells. Flow Cyt (intra): HeLa cells. IP: HeLa whole cell lysate

(ab150035).

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

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.

Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

Protein A purified **Purity**

Clonality Monoclonal
Clone number EPR3472
Isotype IqG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab79398 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)		1/80. For unpurified use at 1/50. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (2)	1/100 - 1/250.
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 70 kDa (predicted molecular weight: 70 kDa). For unpurified use at 1/2000 - 1/5000.
IP	****(1)	1/10 - 1/20.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

Target

Function

Plays an essential role in several cellular processes in DNA metabolism including replication, recombination and DNA repair. Binds and subsequently stabilizes single-stranded DNA intermediates and thus prevents complementary DNA from reannealing.

Functions as component of the alternative replication protein A complex (aRPA). aRPA binds single-stranded DNA and probably plays a role in DNA repair; it does not support chromosomal DNA replication and cell cycle progression through S-phase. In vitro, aRPA cannot promote efficient priming by DNA polymerase alpha but supports DNA polymerase delta synthesis in the presence of PCNA and replication factor C (RFC), the dual incision/excision reaction of nucleotide excision repair and RAD51-dependent strand exchange.

Sequence similarities

Belongs to the replication factor A protein 1 family.

Post-translational modifications

Phosphorylated upon DNA damage, probably by ATM or ATR.

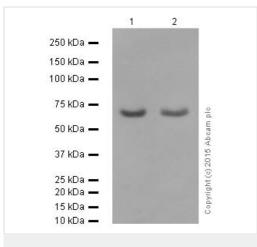
Sumoylated on lysine residues Lys-449 and Lys-577, with Lys-449 being the major site. Sumoylation promotes recruitment of RAD51 to the DNA damage foci to initiate DNA repair

through homologous recombinaison. Desumoylated by SENP6.

Cellular localization

Nucleus.

Images



Western blot - Anti-RPA70 antibody [EPR3472] (ab79398)

All lanes : Anti-RPA70 antibody [EPR3472] (ab79398) at 1/4000 dilution (purified)

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

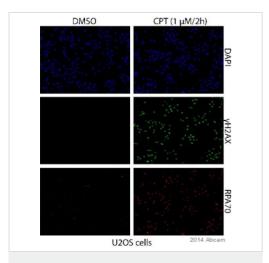
All lanes : Peroxidase-conjugated goat anti-rabbit lgG, (H+L) at 1/1000 dilution

Predicted band size: 70 kDa **Observed band size:** 70 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

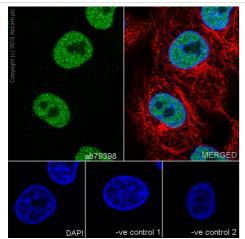
Unpurified ab79398 staining RPA70 in U-2 OS (Human bone osteosarcoma epithelial cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence).

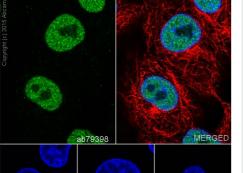
Cells were fixed with paraformaldehyde, permeabilized with 0.5% Triton X-100 in PBS and blocked with 2% BSA for 1 hour at 25°C. Samples were incubated with primary antibody (1/500 in PBS + 0.5% Tween-20) for 2 hours at 25°C. A Cy3®-conjugated goat antirabbit IgG monoclonal (1/250) was used as the secondary antibody.



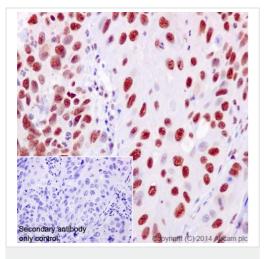
Immunocytochemistry/ Immunofluorescence - Anti-RPA70 antibody [EPR3472] (ab79398)

This image is courtesy of an Abreview submitted by Remi Buisson





Immunocytochemistry/ Immunofluorescence - Anti-RPA70 antibody [EPR3472] (ab79398)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RPA70 antibody [EPR3472] (ab79398)

Immunocytochemistry/Immunofluorescence analysis of A549 (Human lung carcinoma cell line) cells labeling RPA70 with purified ab79398 at 1/200.

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit lgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594conjugated goat anti-mouse IgG (1/500) were also used.

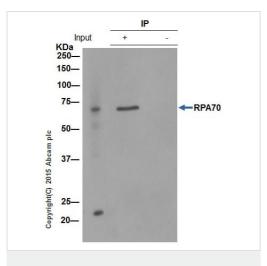
Control 1: primary antibody (1/200) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG (1/500).

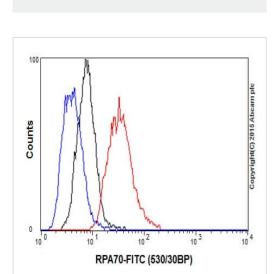
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labeling RPA70 with purified ab79398 at 1/100.

Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500).

Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunoprecipitation - Anti-RPA70 antibody [EPR3472] (ab79398)



Flow Cytometry (Intracellular) - Anti-RPA70 antibody [EPR3472] (ab79398)

ab79398 (purified) at 1/20 immunoprecipitating RPA70 in HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate.

Lane 1 (input): HeLa whole cell lysate (10 µg)

Lane 2 (+): ab79398 + HeLa whole cell lysate (10 μg).

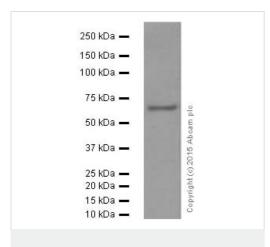
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab79398 in HeLa whole cell lysate.

For western blotting, an HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody (1/1500).

Blocking/Dilution buffer: 5% NFDM/TBST.

Intracellular Flow Cytometry analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling RPA70 with purified ab79398 at 1/80 (red).

Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabeled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-RPA70 antibody [EPR3472] (ab79398)



Western blot - Anti-RPA70 antibody [EPR3472] (ab79398)

Anti-RPA70 antibody [EPR3472] (ab79398) at 1/1000 dilution (purified) + A549 (Human lung carcinoma cell line) cell lysate at 20 µq

Secondary

Peroxidase-conjugated goat anti-rabbit lgG, (H+L) at 1/1000 dilution

Predicted band size: 70 kDa
Observed band size: 70 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-RPA70 antibody [EPR3472] (ab79398) at 1/5000 dilution (unpurified)

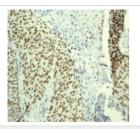
Lane 1 : A549 (Human lung carcinoma cell line) cell lysateLane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP-conjugated goat anti-rabbit lgG at 1/2000 dilution

Predicted band size: 70 kDa **Observed band size:** 70 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RPA70 antibody
[EPR3472] (ab79398)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical squamous cell carcinoma labelling RPA70 with unpurified ab79398 at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

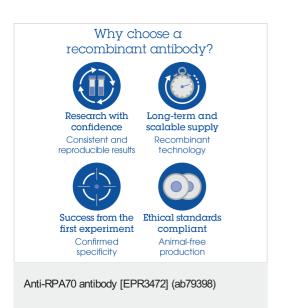
Flow Cytometry (Intracellular) - Anti-RPA70 antibody [EPR3472] (ab79398)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with unpurifiedab79398 (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 (unpurified ab79398, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) $(1\mu g/1x10^6\ cells)\ used\ under\ the\ same\ conditions.$

Acquisition of >5,000 events was performed.



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