abcam

ab157203

Anti-CDK5RAP3 antibody [EPR11294(B)]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR11294(B)
Purification technique	Affinity purification Protein A
Specificity	The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.
Concentration	0.14 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/50 - 1/100

Notes	The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.

Expected

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

IP

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	-

Expected

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

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/10000
Notes	-

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

ICC/IF

Not recommended

Species	Mouse, Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

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

Target data

See full target information CDK5RAP3

Function

Substrate adapter of E3 ligase complexes mediating ufmylation, the covalent attachment of the ubiquitin-like modifier UFM1 to substrate proteins, and which is involved in various processes, such as ribosome recycling and reticulophagy (also called ER-phagy) (PubMed:23152784, PubMed:30635284, PubMed:32851973, PubMed:36121123, PubMed:36543799, PubMed:37595036, PubMed:38383785, PubMed:38383789). As part of the UREL complex, plays a key role in ribosome recycling by promoting mono-ufmylation of RPL26/uL24 subunit of the 60S ribosome (PubMed:38383785, PubMed:38383789). Ufmylation of RPL26/uL24 occurs on free 60S ribosomes following ribosome dissociation: it weakens the junction between post-termination 60S subunits and SEC61 translocons, promoting release and recycling of the large ribosomal subunit from the endoplasmic reticulum membrane (PubMed:38383785, PubMed:38383789). Ufmylation of RPL26/uL24 and subsequent 60S ribosome recycling either take place after normal termination of translation or after ribosome stalling during cotranslational translocation at the endoplasmic reticulum (PubMed:32851973, PubMed:37595036, PubMed:38383785, PubMed:38383789). Within the UREL complex, CDK5RAP3 acts as a substrate adapter that constrains UFL1 ligase activity to mono-ufmylate RPL26/uL24 at 'Lys-134' (PubMed:36121123, PubMed:38383785, PubMed:38383789). The UREL complex is also involved in reticulophagy in

response to endoplasmic reticulum stress by promoting ufmylation of proteins such as CYB5R3, thereby promoting lysosomal degradation of ufmylated proteins (PubMed:36543799). Also acts as a regulator of transcription: negatively regulates NF-kappa-B-mediated gene transcription through the control of RELA phosphorylation (PubMed:17785205, PubMed:20228063). Also regulates mitotic G2/M transition checkpoint and mitotic G2 DNA damage checkpoint (PubMed:15790566, PubMed:19223857). Through its interaction with CDKN2A/ARF and MDM2 may induce MDM2-dependent p53/TP53 ubiquitination, stabilization and activation in the nucleus, thereby promoting G1 cell cycle arrest and inhibition of cell proliferation (PubMed:16173922). May also play a role in the rupture of the nuclear envelope during apoptosis (PubMed:23478299). May regulate MAPK14 activity by regulating its dephosphorylation by PPM1D/WIP1 (PubMed:21283629). Required for liver development (By similarity). (Microbial infection) May be negatively regulated by hepatitis B virus large envelope protein mutant pre-s2 to promote mitotic entry.

Storage

Shipped at conditions	Conditional Ambient
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

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.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

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

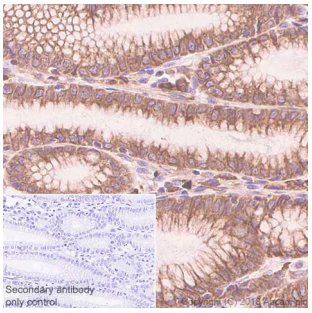
We do not recommend this combination. It is not covered by our product promise.

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.

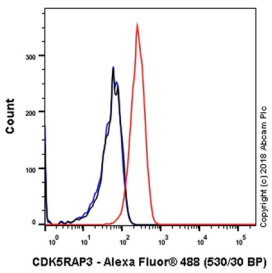
10 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

CDK5RAP3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CDK5RAP3 antibody

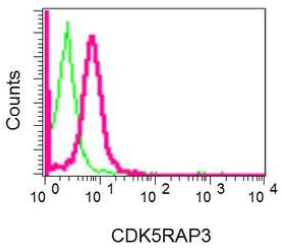
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human stomach tissue sections labeling CDK5RAP3 with purified ab157203 at 1/50 dilution (2.8 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Flow Cytometry (Intracellular) - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

CDK5RAP3 Flow Cytometry (Intracellular) staining using rabbit Anti-CDK5RAP3 antibody

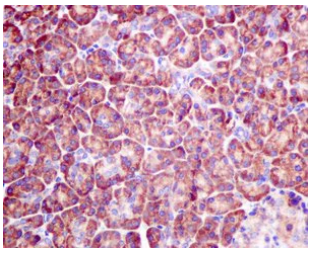
Intracellular Flow Cytometry analysis of HEK-293 (Human embryonic kidney epithelial cell) cells labeling CDK5RAP3 with purified ab157203 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Flow Cytometry (Intracellular) - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

CDK5RAP3 Flow Cytometry (Intracellular) staining using rabbit Anti-CDK5RAP3 antibody

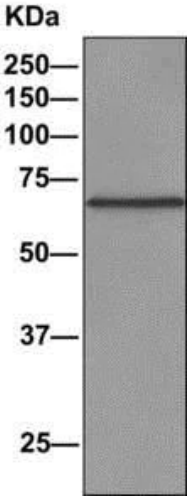
Intracellular Flow Cytometry analysis of permeabilized HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cells labeling CDK5RAP3 (red), using ab157203 (unpurified) at a 1/10 dilution, and negative control cells probed with a Rabbit IgG (green)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

CDK5RAP3 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CDK5RAP3 antibody

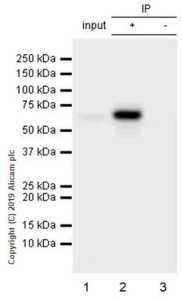
Immunohistochemical analysis of formalin-fixed, paraffin-embedded human pancreas tissue, labeling CDK5RAP3 using ab157203 (unpurified) at a 1/50 dilution.
Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

Western blot analysis of Immunoprecipitation pellet from HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate showing CDK5RAP3, using ab157203 (unpurified) at 1/10 dilution.
All lanes:
Immunoprecipitation - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

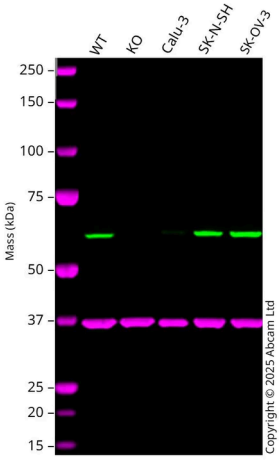
Predicted band size: 57 kDa



Immunoprecipitation - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

ab157203 (purified) at 1/20 dilution (1ug) immunoprecipitating CDK5RAP3 in HEK-293 whole cell lysate. HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10ug
Lane 2 (+): ab157203 & HEK-293 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab157203 in HEK-293 whole cell lysate
For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.
All lanes:
Immunoprecipitation - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

Predicted band size: 57 kDa



Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

CDK5RAP3 Western blot staining using rabbit Anti-CDK5RAP3 antibody

Western blot: Anti-CDK5RAP3 antibody [EPR11294(B)] ab157203 staining at 1/2000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 62 kDa in Wild-type A549 cell lysates with no signal observed at this size in CDK5RAP3 knockout A549 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203) at 1/2000 dilution

Lane 1:
Wild-type A549 at 20 µg

Lane 2:
CDK5RAP3 knockout A549 at 20 µg

Lane 3:
Calu-3 at 20 µg

Lane 4:
SK-N-SH at 20 µg

Lane 5:
SK-OV-3 at 20 µg

Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

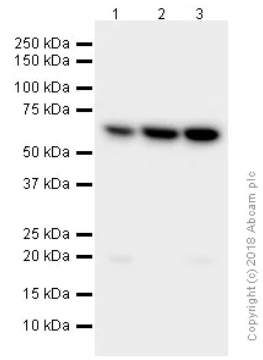
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 57 kDa

Observed band size: 62 kDa, 37 kDa

Exposure time: 60s



Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

All lanes:
Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203) at 1/2000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysates at 20 µg

Lane 2:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 20 µg

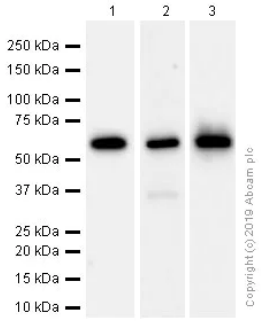
Lane 3:
A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 57 kDa

Observed band size: 57 kDa



Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

All lanes:
Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203) at 1/1000 dilution

Lane 1:
CTLL-2 (Mouse T lymphocyte) whole cell lysates at 20 µg

Lane 2:
EL4 (Mouse lymphoma T lymphocyte) whole cell lysates at 20 µg

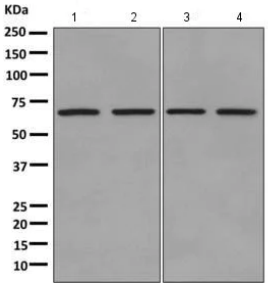
Lane 3:
H9 (Human lymphoma T lymphocyte) whole cell lysates at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 57 kDa

Observed band size: 57 kDa



Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203)

All lanes:
Western blot - Anti-CDK5RAP3 antibody [EPR11294(B)] (ab157203) at 1/1000 dilution

Lane 1:
Jurkat (Human T cell leukemia cell line from peripheral blood) cell lysate at 10 µg

Lane 2:

HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

Lane 3:
A431 (Human epidermoid carcinoma cell line) cell lysate at 10 µg

Lane 4:
HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cell lysate at 10 µg

Predicted band size: 57 kDa

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