abcam

ab199376

Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free)

Recombinant | RabMAb | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Carrier free	Yes
Clone number	EPR25A
Purification technique	Affinity purification Protein A
Concentration	0.99 - 1.032 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Endotoxin level: <1EU/mg (based on Lumulus Amebocyte lysate assay).

Reactivity data

Application	ChIC/CUT&RUN-seq
Reactivity	Reacts
Dilution info	-
Notes	-

Application	IP

Reactivity	Reacts
Dilution info	-
Notes	-

Application	ICC/IF
Reactivity	Reacts
Dilution info	-
Notes	**Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used.

Application	IHC-P
Reactivity	Reacts
Dilution info	-
Notes	**Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application	Flow Cyt
Reactivity	Reacts
Dilution info	1/70
Notes	**Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used.

Application	ChIP-seq
Reactivity	Expected
Dilution info	-
Notes	-

Application	WB
Reactivity	Reacts
Dilution info	-
Notes	-

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

ab199376 is a carrier-free antibody designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes.

KLH is often used in molecular immunology as a carrier protein conjugated to low molecular weight molecules such as peptides, amino acids, nucleic acids, drugs or toxins to render them more immunogenic due to the size of the conjugate complex and the immunogenicity of KLH.

Immunogen
Chemical / Small Molecule conjugated to keyhole limpet haemocyanin. KLH is a copper containing oxygen carrier occurring freely dissolved in the hemolymph of many molluscs and arthropods. KLH forms a large complex composed of ~50 kDa subunits.

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.

What does low endotoxin mean?
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

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.

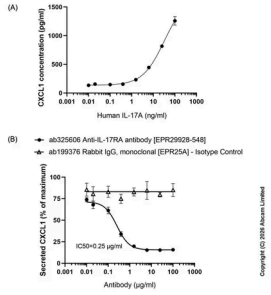
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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11 product images



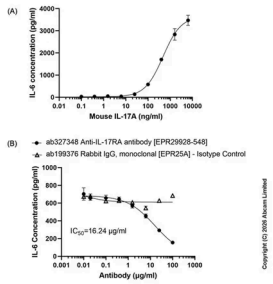
Functional Studies - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

CXCL1 secretion in HT-29 cells following stimulation with IL-17A (Abcam, ab282392) in the presence of either an anti-IL-17RA antibody (ab325606) or an IgG isotype control (ab199376).

(A) Dose–response curve of HT-29 cells exposed to increasing concentrations of IL-17A.

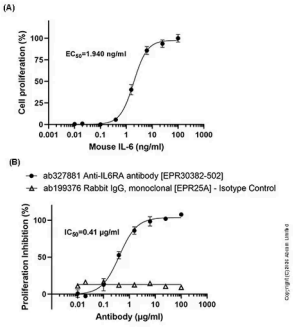
(B) CXCL1 secretion induced by 100 ng/mL human IL-17A following treatment with anti-IL-17RA antibody (circles, solid line) or IgG isotype control (triangles, dotted line) across a range of concentrations. The IC_{50} for inhibition of CXCL1 secretion is 0.25 µg/mL.

HT-29 cells were seeded at 1×10^4 cells per well in a 96-well plate. Cells were pre-incubated with a dilution series of anti-IL-17RA antibody for 6 hours prior to IL-17A stimulation and then cultured for 48 hours in medium containing 10% FBS. CXCL1 levels were measured using a Human CXCL1 (GROα) ELISA kit (Abcam, ab190805).



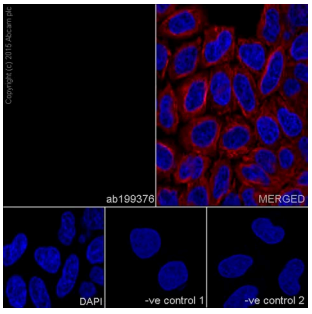
Functional Studies - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

IL-6 secretion in NIH/3T3 cells following stimulation with IL-17A in the presence of either an anti-IL17RA antibody (ab327248) or an IgG isotype control (ab199376). (A) Dose–response curve of NIH/3T3 cells exposed to increasing concentrations of IL-17A. (B) IL-6 secretion induced by 1000 ng/mL mouse IL-17A following treatment with anti-IL17RA antibody(circles, solid line) or IgG isotype control (triangles, dotted line) across a range of concentrations. The IC_{50} for inhibition of IL-6 secretion is 16.24 µg/mL. NIH/3T3 cells were seeded at 1 × 10^4cells per well in a 96-well plate. Cells were pre-incubated with a dilution series of anti-IL17RA antibody for 6 hours prior to IL-17A stimulation and then cultured for 48 hours in medium containing 10% FBS. IL-6 concentrations were measured using a Mouse IL-6 ELISA Kit (Abcam, ab222503).



Functional Studies - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

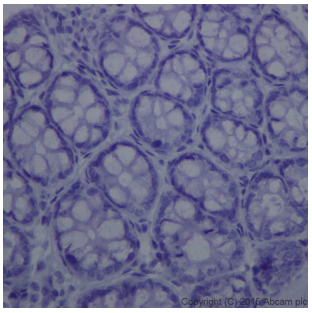
Proliferation inhibition of B9 cells following stimulation with mouse IL-6 in the presence of either an anti-IL6RA antibody (ab327881) or an IgG isotype control (ab199376).(A) Dose–response curve of cell proliferation of B9 cells exposed to increasing concentrations of mouse IL-6.(B) Proliferation inhibition induced by 100 ng/mL mouse IL-6 following treatment with anti-IL6RA antibody (circles, solid line) or IgG isotype control (triangles, dotted line) across a range of concentrations. The IC_{50} for inhibition of proliferation is 0.41 µg/mL.B9 cells were seeded at 3 × 10^4 cells per well in a 96-well plate. Cells were pre-incubated with a dilution series of anti-IL6RA antibody for 6 hours prior to IL-6 stimulation and then cultured for 48 hours in medium containing 5% FBS. Proliferation was analysed by luminescent assay and recorded using a Microplate Reader at 560nm emission.



Immunocytochemistry/ Immunofluorescence - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

Immunocytochemistry/ Immunofluorescence staining using rabbit Anti- antibody

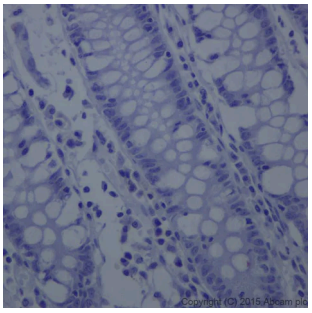
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeled with ab199376 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution. Confocal image showing no staining on HeLa cell line. 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 1/1000 dilution (red).
The negative controls are as follows:-
-ve control 1: ab199376 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 IgG H&L) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

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

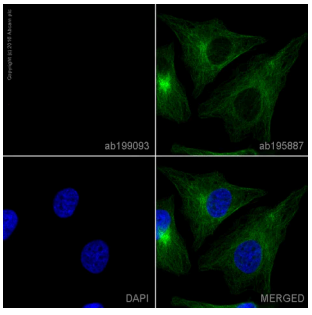
Immunohistochemistry analysis of rat colon tissue control for testing ab199376 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. An anti-rabbit IgG H&L (HRP) (ab97051) was used as a secondary antibody. Counterstained with hematoxylin. No staining on rat colon tissue was observed.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

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

Immunohistochemistry analysis of human colon tissue control for testing ab199376 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. An anti-rabbit IgG H&L (HRP) (ab97051) was used as a secondary antibody. Counterstained with hematoxylin. No staining on human colon tissue was observed.



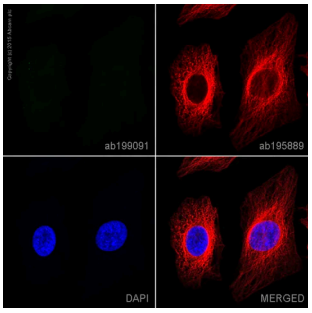
Immunocytochemistry/ Immunofluorescence - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

Immunocytochemistry/ Immunofluorescence staining using rabbit Anti- antibody

Clone EPR25A (ab199376) has been successfully conjugated by Abcam. This image was generated using Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 647). Please refer to ab199093 for protocol details.
Immunofluorescent analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells, fixed with 4% formaldehyde (10 min). The cells were permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab199093 (Rabbit IgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labeled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).



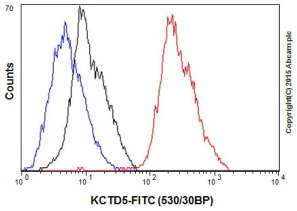
Immunocytochemistry/ Immunofluorescence - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

Immunocytochemistry/ Immunofluorescence staining using rabbit Anti- antibody

Clone EPR25A (ab199376) has been successfully conjugated by Abcam. This image was generated using Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Alexa Fluor® 488). Please refer to ab199091 for protocol details.
Immunofluorescent analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells, fixed with 4% formaldehyde (10 min). The cells were permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab199091 (Rabbit IgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (pseudocolored in red). Nuclear DNA was labeled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

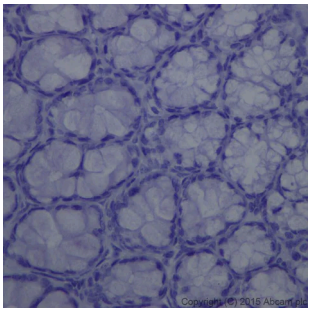
This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).



Flow Cytometry - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

Flow Cytometry staining using rabbit Anti- antibody

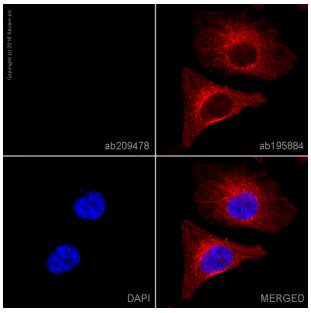
Flow cytometric analysis of 4% paraformaldehyde-fixed Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling KCTD5 with ab194825 at 1/70 dilution (red) compared with ab199376 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody. ab199376 detects no signal in FC.
Note: ab199376 detects no signal in Flow cytometry.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

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

Immunohistochemistry analysis of mouse colon tissue control for testing ab199376 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. An anti-rabbit IgG H&L (HRP) (ab97051) was used as a secondary antibody. Counterstained with hematoxylin. No staining on mouse colon tissue was observed.



Immunocytochemistry/ Immunofluorescence - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

Immunocytochemistry/ Immunofluorescence staining using rabbit Anti- antibody

Clone EPR25A (ab199376) has been successfully conjugated by Abcam. This image was generated using Rabbit IgG, monoclonal [EPR25A] - Isotype Control (PE). Please refer to ab209478 for protocol details.
Immunofluorescent analysis of HeLa (human cervical cancer) cells, fixed with 4% formaldehyde (10 min). The cells were permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab209478 (Rabbit IgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and ab195884, Rat monoclonal [YOL1/34] to Tubulin Microtubule Marker (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).

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