# abcam

## Product datasheet

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

Recombinant RoloWAb

#### 14 References 9 Images

#### Overview

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

**Tested applications** Suitable for: ChIP-sequencing, ChIC/CUT&RUN-seq, IHC-P, Flow Cyt, ICC/IF, IP

**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.

**General notes** ab199376 is a carrier-free antibody designed for use in antibody labeling, including

fluorochromes, metal isotopes, oligonucleotides, enzymes.

Our Low endotoxin, azide-free formats have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional

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.

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**

**Form** 

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Purification notes Endotoxin level: <1EU/mg (based on Lumulus Amebocyte lysate assay).

Clone number Monoclone

EPR25A

**Isotype** IgG

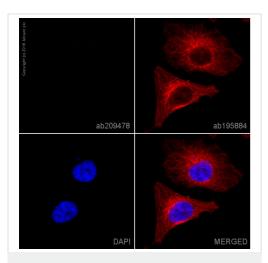
### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab199376 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
ChIP-sequencing		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		1/70.
ICC/IF		1/250.
IP		Use at an assay dependent concentration.

#### **Images**



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

Clone EPR25A (ab199376) has been successfully conjugated by Abcam. This image was generated using Rabbit lgG, monoclonal [EPR25A] - Isotype Control (PE). Please refer to <a href="mailto:ab209478">ab209478</a> 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 <a href="mailto:ab209478">ab209478</a> (Rabbit IgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and <a href="mailto:ab195884">ab195884</a>, 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).

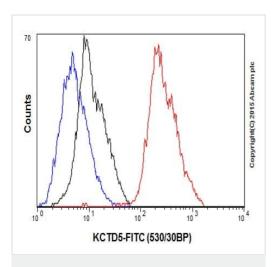
Image was taken with a confocal microscope (Leica-Microsystems,

TCS SP8).

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

Flow cytometric analysis of 4% paraformaldehyde-fixed Jurkat (Human T cell leukemia cells from peripheral blood) cells labeling KCTD5 with <a href="mailto:ab194825">ab194825</a> at 1/70 dilution (red) compared with ab199376 Rabbit lgG, 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 lgG (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.



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

ab199093 ab195887

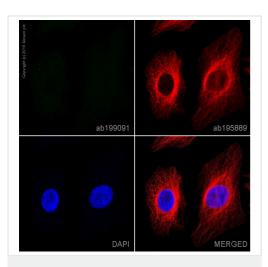
Immunocytochemistry/ Immunofluorescence - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

Clone EPR25A (ab199376) has been successfully conjugated by Abcam. This image was generated using Rabbit lgG, 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 <a href="mailto:ab199093">ab199093</a> (Rabbit lgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and <a href="mailto:ab195887">ab195887</a>, 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)

Clone EPR25A (ab199376) has been successfully conjugated by Abcam. This image was generated using Rabbit lgG, 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 <a href="mailto:ab199091">ab199091</a> (Rabbit lgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and <a href="mailto:ab195889">ab195889</a>, 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).

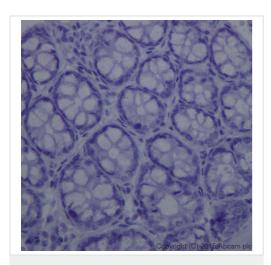
ab199376 MERGED

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

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).

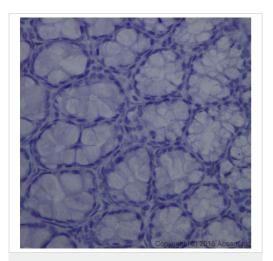
The negative controls are as follows:-

-ve control 1: ab199376 at 1/250 dilution followed by <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.



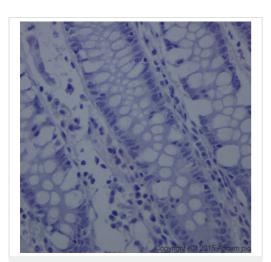
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

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 lgG H&L (HRP) (ab97051) was used as a secondary antibody. Counterstained with hematoxylin. No staining on rat colon tissue was observed.



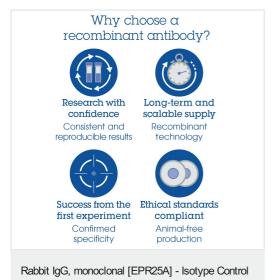
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Rabbit IgG, monoclonal [EPR25A] - Isotype Control (Low endotoxin, Azide free) (ab199376)

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 lgG H&L (HRP) (ab97051) was used as a secondary antibody. Counterstained with hematoxylin. No staining on mouse colon tissue was observed.



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

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 lgG H&L (HRP) (ab97051) was used as a secondary antibody. Counterstained with hematoxylin. No staining on human colon tissue was observed.



(Low endotoxin, Azide free) (ab199376)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

### Our Abpromise to you: Quality guaranteed and expert technical support

• Replacement or refund for products not performing as stated on the datasheet

- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- · We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors