abcam

Product datasheet

Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control ab199091

Recombinant RabMAb

17 References 5 Images

Overview

Product name	Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control
Conjugation	Alexa Fluor® 488. Ex: 495nm, Em: 519nm
Specificity	Please note Abcam have optimised the validation of this product. In our hands, we observe an increase in background signal intensity with the use of Triton X-100 and would recommend using an alternative permeabilisation method such as methanol or saponin.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt, Flow Cyt (Intra)
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	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**. Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or

prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or **outlicensing@thermofisher.com**.

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. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR25A
Isotype	IgG

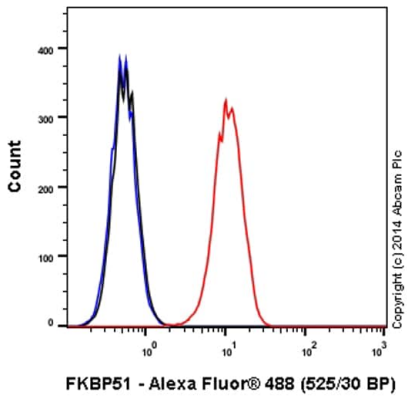
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab199091 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
ICC/IF		1/500.
Flow Cyt		1/500. PubMed: 30937272 **Please note**: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used.
Flow Cyt (Intra)		1/500.

Images

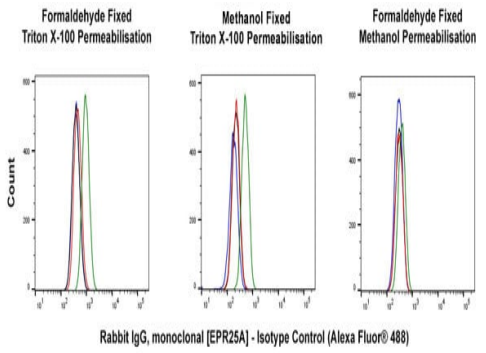


Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with **ab198978** (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 (**ab198978**, 1/500 dilution) for 30 min at 22ºC.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 (ab199091) used at the same concentration and conditions as the primary antibody. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab199091 using various fixation and permeablisation. The cells were fixed, washed and permeablised as indicated below;

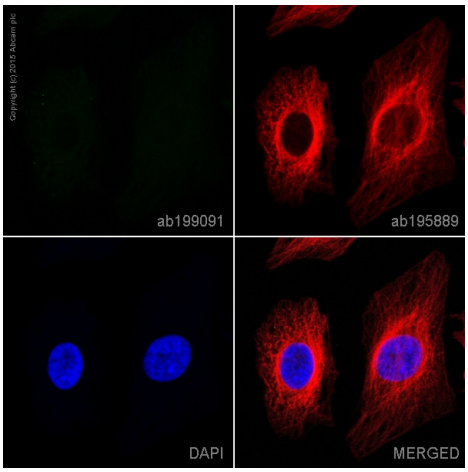
4% formaldehyde (10 min, room temperature)/0.1% PBS-Triton X-100 (15 min, room temperature)

80% methanol (5 min, -20°C)/0.1% PBS-Triton X-100 (15 min, room temperature)

4% formaldehyde (10 min, room temperature)/90% methanol (30 min, -20°C)

The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab199091) for 30 min at 22ºC at the following concentrations - Blue line (Unlabelled), Black line (0.1µg/ml), Red line (1µg/ml) and Green line (10µg/ml) .

Acquisition of >5,000 events were collected using a 50mW Blue laser (488nm) and 530/30 bandpass filter.

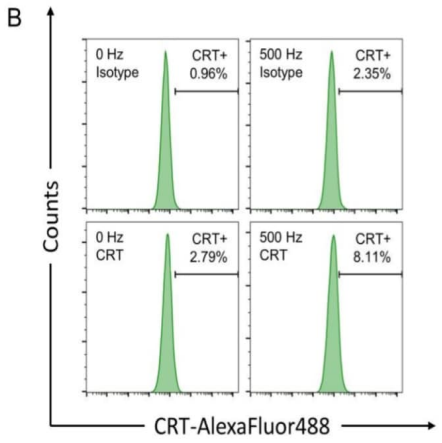


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

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).

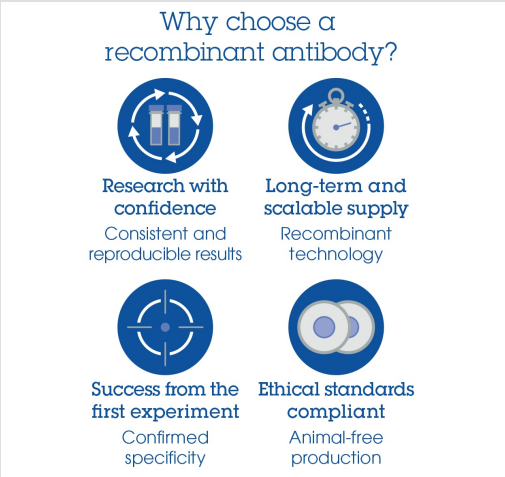
This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).



Flow Cytometry - Alexa Fluor® 488 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199091)

Lin et al Adv Sci (Weinh). 2019 Jan 28;6(6):1802062. doi: 10.1002/advs.201802062. eCollection 2019 Mar 20. Fig S9.

CRT was measured using dual staining of PI and a monoclonal CRT antibody. Following 24 h after incubation, cells were washed with PBS, detached with 200 µL of accutase, and washed twice with 2 mL of FACS buffer (500 mL sheath fluid + 2 g bovine serum albumin + 1 g NaN_3 in 100 mL H_2O). Each sample was split into two vials and one was stained with monoclonal primary rabbit anti-CRT antibody (Abcam, **ab196158**) while the other was stained with rabbit IgG, monoclonal isotype control (Abcam, ab199091) for 40 min at 4°C. Cells were then washed once with FACS buffer. 0.5 µL of PI was added to each sample immediately before being quantified with a flow cytometer. Fifteen thousand events were collected and only the PI− cells were analyzed for surface CRT expression. Data were analyzed and gated using the FlowJo software (FlowJo LLC, version 10). Data were expressed as percent CRT positive after accounting for nonspecific binding with their corresponding isotype.



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