# abcam

# Product datasheet

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

重组 RabMAb

17 References 5 图像

#### 概述

产品名称

Alexa Fluor® 488荧光兔lgG,单克隆抗体[EPR25A] -同型对照

偶联物

Alexa Fluor® 488. Ex: 495nm, Em: 519nm

特异性

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.

经测试应用

适用于: ICC/IF, Flow Cyt, Flow Cyt (Intra)

免疫原

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.

常规说明

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<sup>®</sup> 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 <a href="mailto:outlicensing@thermofisher.com">outlicensing@thermofisher.com</a>.

## 性能

形式 Liquid

存放说明 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.

**存储溶液** pH: 7.40

Preservative: 0.02% Sodium azide

Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA

纯**度** Protein A purified

 克隆
 单克隆

 克隆编号
 EPR25A

#### 应用

同种型

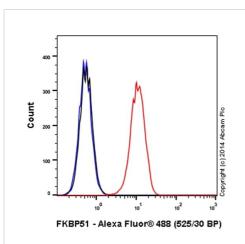
# The Abpromise guarantee Abpromise™承诺保证使用ab199091于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

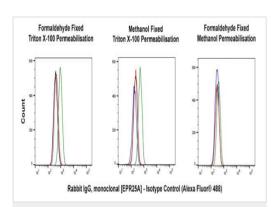
ΙgG

应用	Ab评论	说明
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.

## 图片



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



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 <u>ab198978</u> (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 nonspecific protein-protein interactions followed by the antibody (<u>ab198978</u>, 1/500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor<sup>®</sup> 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.

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;

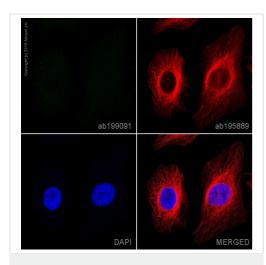
4% formaldehyde (10 min, room temperature)/0.1% PBS-Triton X-100 (15 min, room temperature)

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

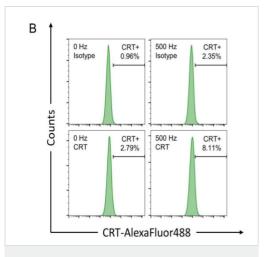
4% formaldehyde (10 min, room temperature)/90% methanol (30 min, -20°C)

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 22°C at the following concentrations - Blue line (Unlabelled), Black line (0.1 $\mu$ g/ml), Red line (1 $\mu$ g/ml) and Green line (10 $\mu$ g/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)



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.

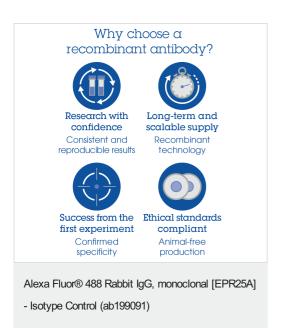
Immunofluorescent analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells, fixed with 4% formaldehyde (10 min). The cells were permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab199091 (Rabbit lgG, 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).

# Reproduced under the Creative Commons license <a href="http://creativecommons.org/licenses/by/4.0/">http://creativecommons.org/licenses/by/4.0/</a>.

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<sub>3</sub> in 100 mL H<sub>2</sub>O). Each sample was split into two vials and one was stained with monoclonal primary rabbit antil CRT antibody (Abcam, ab196158) while the other was stained with rabbit lgG, 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.



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.cn/abpromise">https://www.abcam.cn/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