# abcam

# Product datasheet

# Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control ab199093

重组 RabMAb

14 References 5 图像

概述

产品名称 Alexa Fluor® 647荧光兔lgG,单克隆抗体[EPR25A] -同型对照

Alexa Fluor® 647. Ex: 652nm, Em: 668nm

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

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.

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**.

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## 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

**同种型** IgG

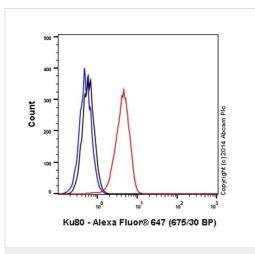
#### 应用

# The Abpromise guarantee Abpromise™承诺保证使用ab199093于以下的经测试应用

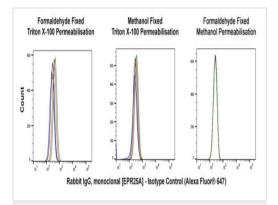
"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.  Please note: This product should be diluted to the same concentration (not dilution) of the primary antibody to be used.

## 图片



Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)



Flow Cytometry - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab199093)

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with <u>ab198587</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>ab198587</u>, 1/50 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor<sup>®</sup> 647 (ab199093) 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 solid-state 25mW red diode laser (635 nm) and 675/30 bandpass filter.

Overlay histogram showing HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained with ab199093 using various fixation and permeablisation. The cells were fixed, washed and permeablised as indicated below;

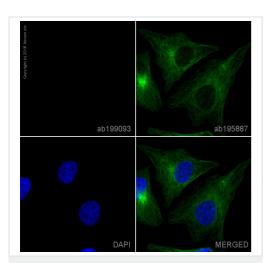
4% formaldehyde (10 min, room temperature)/0.1% PBS-Triton X-100 (15 min, room temperature)

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 (0.01 $\mu$ g/ml), Red line (0.1 $\mu$ g/ml) and Green line (1 $\mu$ g/ml) .

Acquisition of >5,000 events were collected using a 40mW Red laser (640nm) and 670/14 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] -Isotype Control (ab199093)

DAPI EGFR PD-L1 PD-L1/DAPI

Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Rabbit IgG, monoclonal [EPR25A] -Isotype Control (ab199093)

Kulasinghe et al BMC Cancer. 2017 May 16;17(1):333. doi: 10.1186/s12885-017-3316-3. Fig 4.

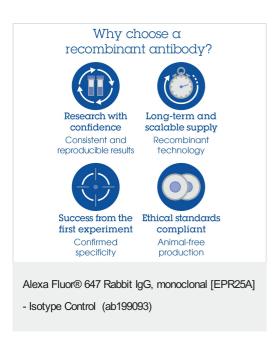
Immunofluorescent analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cells, fixed with 4% formaldehyde (10 min). The cells were permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab199093 (Rabbit lgG, monoclonal [EPR25A] - Isotype Control) at 1/500 dilution (showing no signal) and ab195887, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 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).

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The initial sample was stained using an antibody cocktail and anti-EGFR antibody. A further slide was fixed with 4% formaldehyde for 10 mins, permeabilized with 0.2% Triton X-100 for 5 mins and blocked with 10% fetal-bovine serum in 0.1% PBS-Tween for 1 h at room temperature. The cells were incubated overnight at 4 °C with anti-EGFR antibody and anti-PD-L1 antibody [28-8] (Alexa Fluor® 647) (Abcam <a href="mailto:ab209960">ab209960</a>) 1/100 dilution. Nuclear DNA was visualized with DAPI. Rabbit IgG monoclonal isotype control (Alexa Fluor® 647) (ab199093) was used to identify nonspecific binding. Cells were imaged on the Olympus IX3 inverted microscope.



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

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