# abcam

# Product datasheet

# Anti-Eg5 antibody [EPR23276-52] ab254298



重组 RabMAb

2 References 13 图像

概述

产品名称 Anti-Eg5抗体[EPR23276-52]

描述 兔单克隆抗体[EPR23276-52] to Eg5

宿主 Rabbit

适用于: Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P 经测试应用

不适用于: IHC-Fr

种属反应性 与反应: Mouse, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Human testis and tonsil tissue lysate. Mouse testis tissue lysate. Jurkat, NCI-H1975, MCF7

> and 4T1 whole cell lysate. IHC-P: Human tonsil and testis tissue. Human breast cancer tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa and NIH/3T3 cells. IP: HeLa and NIH/3T3

whole cell lysate.

This product is a recombinant monoclonal antibody, which offers several advantages including: 常规说明

- 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**.

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.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR23276-52

**同种型** IgG

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab254298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
Flow Cyt (Intra)		1/600.
ICC/IF		1/50.
IP		1/30.
WB		1/1000. Predicted molecular weight: 119 kDa.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

应用说明

Is unsuitable for IHC-Fr.

#### 靶标

功能

Motor protein required for establishing a bipolar spindle. Blocking of KIF11 prevents centrosome migration and arrest cells in mitosis with monoastral microtubule arrays.

疾病相关

Defects in KIF11 are the cause of microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR) [MIM:152950]. An autosomal dominant disorder that involves an overlapping but variable spectrum of central nervous system and ocular developmental anomalies. Microcephaly ranges from mild to severe and is often associated with mild to moderate developmental delay and a characteristic facial phenotype with upslanting palpebral fissures, broad nose with rounded tip, long philtrum with thin upper lip, prominent chin, and prominent ears. Chorioretinopathy is the most common eye abnormality, but retinal folds, microphthalmia, and myopic and hypermetropic astigmatism have also been reported, and some individuals have no overt ocular phenotype. Congenital lymphedema, when present, is typically confined to the dorsa of the feet, and lymphoscintigraphy reveals the absence of radioactive isotope uptake from the webspaces between the toes.

序列相似性

Belongs to the kinesin-like protein family. BimC subfamily.

Contains 1 kinesin-motor domain.

翻译后修饰

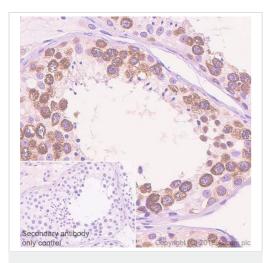
Phosphorylated exclusively on serine during S phase, but on both serine and Thr-926 during mitosis, so controlling the association of KIF11 with the spindle apparatus (probably during early prophase). Phosphorylated upon DNA damage, probably by ATM or ATR.

A subset of this protein primarily localized at the spindle pole is phosphorylated by NEK6 during

mitosis; phosphorylation is required for mitotic function.

细胞定位

Cytoplasm. Cytoplasm > cytoskeleton > spindle pole.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Eg5 antibody
[EPR23276-52] (ab254298)

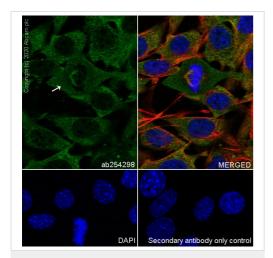
Immunohistochemical analysis of paraffin-embedded human testis tissue labeling Eg5 with 254298 at 1/1/4000 (0.17µg/ml) followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Cytoplasmic staining in spermatocytes of human testis is observed.

The section was incubated with ab254298 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

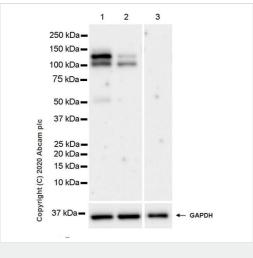
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Eg5 antibody [EPR23276-52] (ab254298)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 cells labeling Eg5 with ab254298 at 1/50 dilution, followed by  $\underline{ab150077}$  Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2  $\mu$ g/ml dilution (Green). Confocal image showing cytoplasmic and spindle (arrow) staining in NIH/3T3 cells.  $\underline{ab195889}$  Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5  $\mu$ g/ml dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) at  $1/1000\ 2\ \mu g/ml$  dilution.



Western blot - Anti-Eg5 antibody [EPR23276-52] (ab254298)

**All lanes :** Anti-Eg5 antibody [EPR23276-52] (ab254298) at 1/1000 dilution

Lane 1: Human testis tissue lysate

Lane 2: Human tonsil tissue lysate

Lane 3: Human lung tissue lysate

Lysates/proteins at 20 µg per lane.

## Secondary

**All lanes :** VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/100000 dilution

Predicted band size: 119 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID:23857769; 27492783).

Negative control: Human lung (PMID: 27279560).

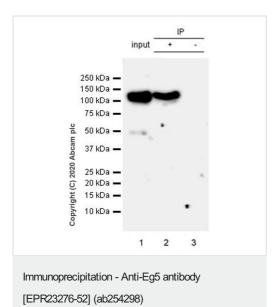
The minor bands beneath the target band may correspond to degradation.

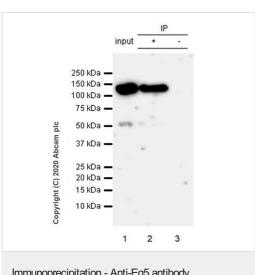
Exposure time: 48 seconds.

Eg5 – Alexa Fluor®488 (530/30BP)

Flow Cytometry (Intracellular) - Anti-Eg5 antibody [EPR23276-52] (ab254298)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labeling Eg5 with ab254298 at 1/600 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.





Immunoprecipitation - Anti-Eg5 antibody [EPR23276-52] (ab254298)

Eg5 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 ug with ab254298 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254298 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: NIH/3T3 whole cell lysate 10 ug.

Lane 2: ab254298 IP in NIH/3T3 whole cell lysate.

**Lane 3:** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab254298 in NIH/3T3 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes

Fresh lysates were used in this IP.

Eg5 was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate 10 ug with ab254298 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254298 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 ug.

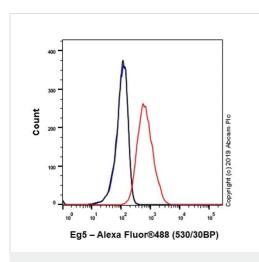
Lane 2: ab254298 IP in HeLa whole cell lysate.

**Lane 3:** Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab254298 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

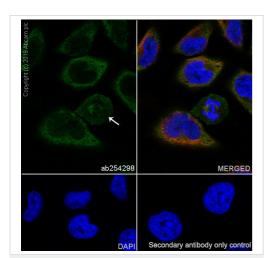
Exposure time: 3 minutes.

Fresh lysates were used in this IP.



Flow Cytometry (Intracellular) - Anti-Eg5 antibody [EPR23276-52] (ab254298)

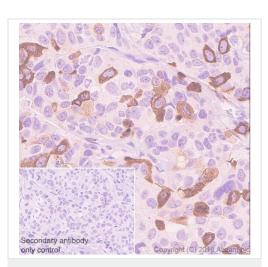
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Eg5 with ab254298 at 1/600 dilution (0.1µg) (Red) compared with a Rabbit monoclonal lgG (ab172730) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Eg5 antibody [EPR23276-52] (ab254298)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Eg5 with ab254298 at 1/50 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) antibody at 1/1000 2  $\mu$ g/ml dilution (Green). Confocal image showing cytoplasmic and spindle (arrow) staining in HeLa cells. <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 2.5  $\mu$ g/ml dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) at  $1/1000 \ 2 \ \mu g/ml$  dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Eg5 antibody
[EPR23276-52] (ab254298)

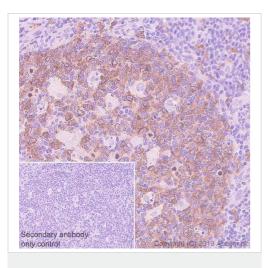
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling Eg5 with ab254298 at 1/4000 (0.17µg/ml) followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Scattered cytoplasmic staining in human breast cancer cells is observed (PMID:29181100).

The section was incubated with ab254298 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Eg5 antibody
[EPR23276-52] (ab254298)

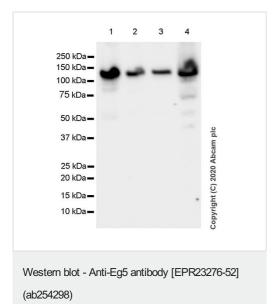
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Eg5 with 254298 at 1/4000 (0.17 $\mu$ g/ml) followed by a ready to use Goat Anti-Rabbit lgG H&L (HRP). Cytoplasmic staining in human tonsil is observed (PMID:25277178). The section was incubated with ab254298 for 30 mins at room

The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with hematoxylin.

temperature.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit lgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



**All lanes :** Anti-Eg5 antibody [EPR23276-52] (ab254298) at 1/1000 dilution

**Lane 1**: Jurkat (human T cell leukemia T lymphocyte), whole cell lvsate

Lane 2: NCI-H1975 (human adenocarcinoma lung epithelial cell)

Lane 3: MCF7 (human breast adenocarcinoma epithelial cell)

Lane 4: 4T1 (mouse mammary gland carcinoma epithelial cell)

Lysates/proteins at 20 µg per lane.

## Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 119 kDa

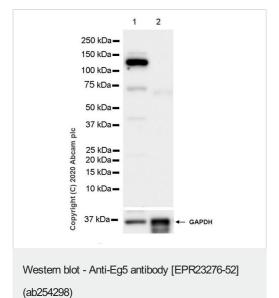
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID:23857769; 27492783).

#### Fresh lysates were tested in this WB.

The minor bands beneath the target band in lane 1 and lane 4 may correspond to degradation.

Exposure time: 15 seconds.



**All lanes :** Anti-Eg5 antibody [EPR23276-52] (ab254298) at 1/1000 dilution

Lane 1 : Mouse testis tissue lysate

Lane 2 : Mouse brain tissue lysate

Lysates/proteins at 20 µg per lane.

# **Secondary**

**All lanes :** Goat Anti-Rabbit  $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$  at 1/100000 dilution

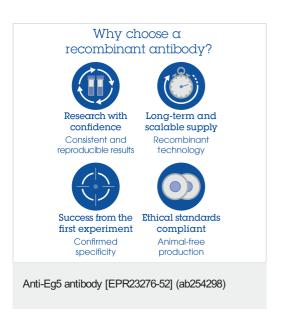
Predicted band size: 119 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: Mouse brain (PMID: 17974955).

The minor bands beneath the target band may correspond to degradation.

Exposure time: 48 seconds.



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