abcam

Product datasheet

Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] ab205606

重组 RabMAb

★★★★★ 1 Abreviews 67 References 11 图像

概述

产品名称 Anti-DDDDK tag (Binds to FLAG® tag sequence)抗体[EPR20018-251]

描述 兔单克隆抗体[EPR20018-251] to DDDDK tag (Binds to FLAG® tag sequence)

宿主 Rabbit

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

种属反应性 与反应: Species independent

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: HEK-293 transfected with PFKFB3 and HEK-293 transfected with PD-L1 cell lysate. IHC-P:

HEK-293T transfected with DDDDK-tagged human PD-L1. ICC/IF: HEK-293T cells. Flow: HEK-293T transfected with DDDDK-tagged human PD-L1. IP: HEK-293T transfected with DDDDK-

tagged human PFKFB3.

常规说明 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**.

 $\mathsf{FLAG}^{\texttt{B}}$ is a registered trade mark of Sigma Aldrich Biotechnology LP. It is used here for

informational purposes only.

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

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

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR20018-251

同种型 IgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab205606于以下的经测试应用

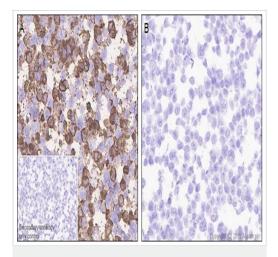
"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (1)	1/500 - 1/5000. This antibody can be used at 1.2 μg/ml. ab205606 can detect extra bands in non-transfected cell lines at low dilution.
ICC/IF		1/100.
Flow Cyt		1/700.
IHC-P		1/750. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

相关性 This is a useful tool for the localisation and characterisation of DDDDK tagged proteins (Binds to

FLAG® tag sequence).

图片

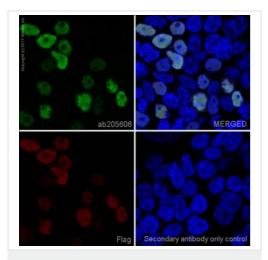


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Immunhistochemical analysis of agarose-embedded HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with DDDDK-tagged human PD-L1 expression vector labeling DDDDK tag with ab205606 at 1/750 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Counterstained with hematoxylin.

Positive staining on HEK-293T cells transfected with DDDDK-tagged human PD-L1 expression vector (Panel A) is observed. No signal was detected on HEK-293T transfected with an empty vector (vector control), containing a C-terminal DDDDK tag (Panel B).

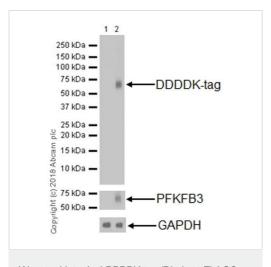
Heat mediated antigen retrieval was performed using EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) cells labeling DDDDK tag with ab205606 at 1/100 dilution, followed by **ab150077** AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 dilution (green).

Confocal image showing positive staining for FLAG® on HEK-293T cells transfected with DDDDK-tagged PFKFB3 expression vector. Mouse monoclonal ANTI-FLAG® M2 antibody was used as a counterstain at 1/500 dilution, and AlexaFluor®647 Goat anti-mouse secondary (ab150115) was used as the secondary antibody only control at 1/500 dilution. The nucleus is counterstained with DAPI.



Western blot - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

All lanes : Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1/1000 dilution

Lane 1: HEK-293 (human epithelial cell line from embryonic kidney) transfected with an empty vector (vector control), containing an N-terminal DDDDK tag, whole cell lysate

Lane 2: HEK-293 transfected with PFKFB3 (WT) expression vector containing an N-terminal DDDDK tag, whole cell lysate

Lysates/proteins at 10 µg per lane.

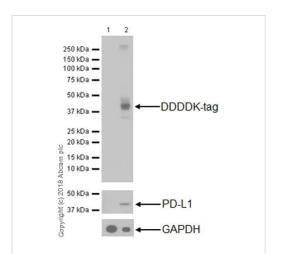
Secondary

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

Observed band size: 60 kDa

Blocking/Diluting buffer: 5% NFDM/TBST

Exposure time: 0.5 seconds



Western blot - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

All lanes : Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1/5000 dilution

Lane 1 : HEK-293 (human epithelial cell line from embryonic kidney) transfected with an empty vector (vector control), containing a C-terminal DDDDK tag, whole cell lysate

Lane 2: HEK-293 transfected with PD-L1 (WT) expression vector containing a C- terminal DDDDK tag, whole cell lysate

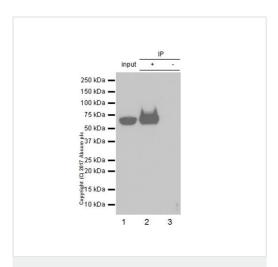
Lysates/proteins at 10 µg per lane.

Secondary

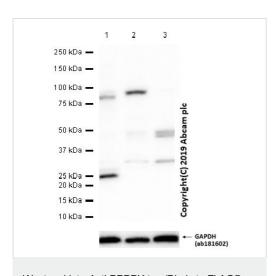
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Blocking/Diluting buffer: 5% NFDM/TBST

Exposure time: 1 second



Immunoprecipitation - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)



Western blot - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

DDDDK tag was immunoprecipitated from 0.35 mg HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with DDDDK-tagged human PFKFB3 expression vector whole cell lysate with ab205606 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab205606 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: HEK-293T transfected with DDDDK-tagged human PFKFB3 expression vector whole cell lysate (input).

Lane 2: ab205606 IP in HEK-293T transfected with DDDDK-tagged human PFKFB3 expression vector whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab205606 in HEK-293T transfected with DDDDK-tagged human PFKFB3 expression vector whole cell lysate.

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 3 seconds.

All lanes: Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1.2 µg/ml (1:500 dilution)

Lane 1 : HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2: NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

Lane 3: C6 (rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Developed using the ECL technique.

Observed band size: 25,30,45,80 kDa

Exposure time: 20 seconds

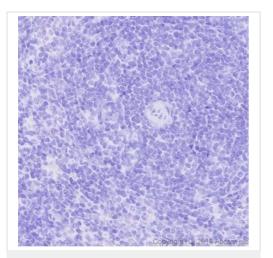
Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

ab205606 can detect extra bands in non-transfected cell lines at low dilution.

DDDDK tag- Alexa Fluor® 488 (530/30 BP)

Flow Cytometry - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Flow cytometric analysis of of 4% paraformaldehyde-fixed, 90% methanol permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with DDDDK-tagged human PD-L1 expression vector labeling DDDDK tag with ab205606 at 1/700 dilution (Red) compared with the Rabbit monoclonal lgG isotype control (ab172730) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077), at 1/2000 dilution was used as the secondary antibody.

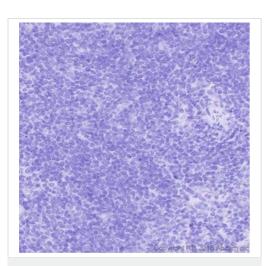


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Negative control: No staining on rat spleen.

Immunohistochemical analysis of paraffin-embedded rat spleen tissue stained for DDDDK tag using ab205606 at 1/750 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Counterstained with hematoxylin.

Heat mediated antigen retrieval was performed using EDTA buffer pH 9 before commencing with IHC staining protocol.

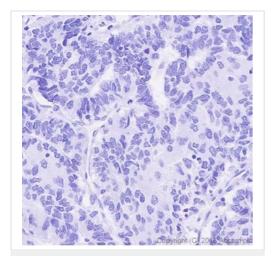


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Negative control: No staining on mouse spleen.

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue stained for DDDDK tag using ab205606 at 1/750 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Counterstained with hematoxylin.

Heat mediated antigen retrieval was performed using EDTA buffer pH 9 before commencing with IHC staining protocol.

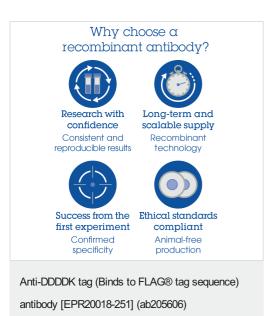


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Negative control: No staining on human hepatocellular cancer.

Immunohistochemical analysis of paraffin-embedded human hepatocellular cancer tissue stained for DDDDK tag using ab205606 at 1/750 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Counterstained with hematoxylin.

Heat mediated antigen retrieval was performed using EDTA buffer pH 9 before commencing with IHC staining protocol.



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