

Product datasheet

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

Recombinant RabMAb

★★★★★ [1 Abreviews](#) [67 References](#) [11 Images](#)

Overview

Product name	Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251]
Description	Rabbit monoclonal [EPR20018-251] to DDDDK tag (Binds to FLAG® tag sequence)
Host species	Rabbit
Tested applications	Suitable for: WB, ICC/IF, Flow Cyt, IHC-P, IP
Species reactivity	Reacts with: Species independent
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.</p> <p>FLAG® is a registered trade mark of Sigma Aldrich Biotechnology LP. It is used here for informational purposes only.</p>

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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide

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

Purity Protein A purified
Clonality Monoclonal
Clone number EPR20018-251
Isotype IgG

Applications

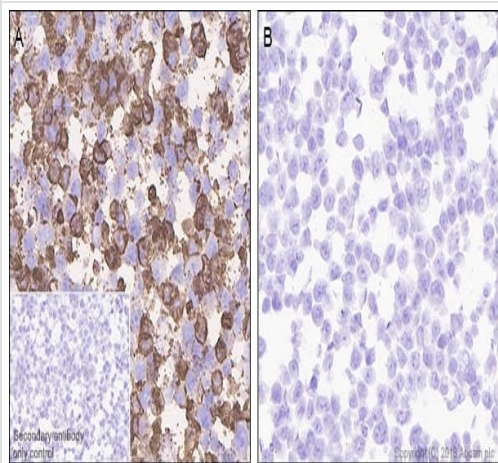
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab205606 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
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.

Target

Relevance This is a useful tool for the localisation and characterisation of DDDDK tagged proteins (Binds to FLAG® tag sequence).

Images

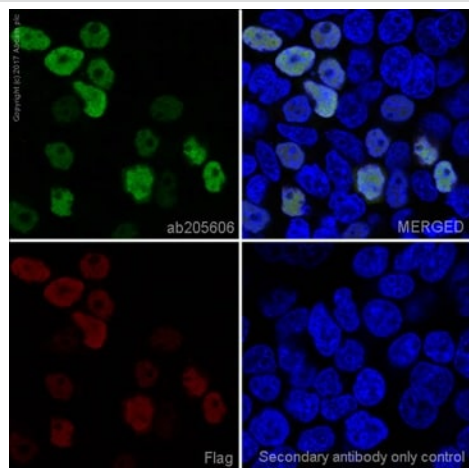


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

Immunohistochemical 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).

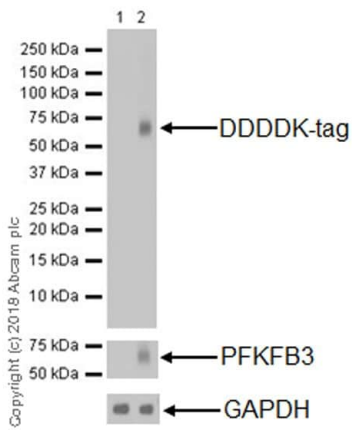
Heat mediated antigen retrieval was performed using EDTA buffer pH 9 before commencing with IHC staining protocol.



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.

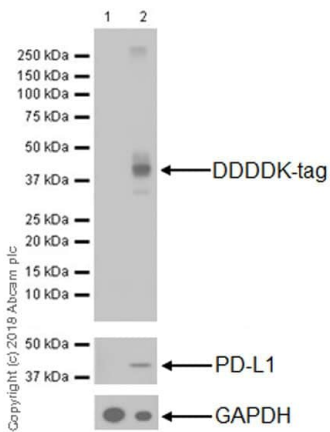
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) 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

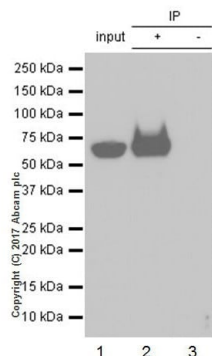
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG 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)

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.

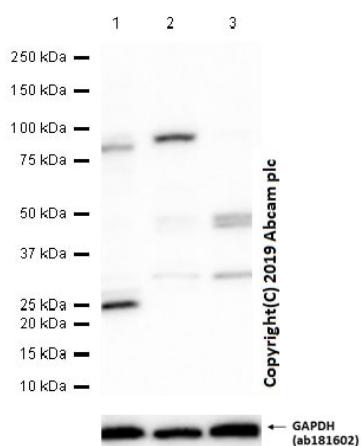
Lane 1: HEK-293T transfected with DDDDK-tagged human PFKFB3 expression vector whole cell lysate (input).

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

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

Blocking/Dilution buffer: 5% NFD/MTBST

Exposure time: 3 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 1.2 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 IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

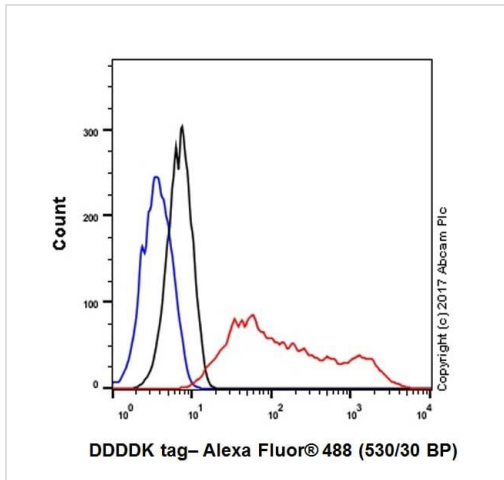
Developed using the ECL technique.

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

Exposure time: 20 seconds

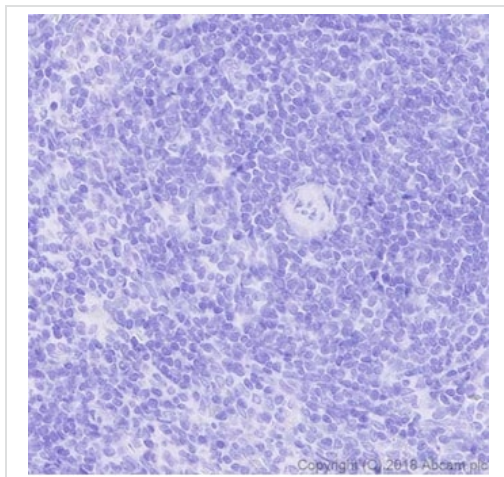
Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

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



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

Flow cytometric analysis 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 IgG isotype control (**ab172730**) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**), at 1/2000 dilution was used as the secondary antibody.

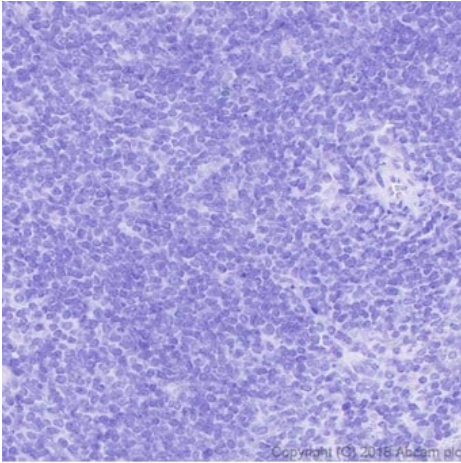


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

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.

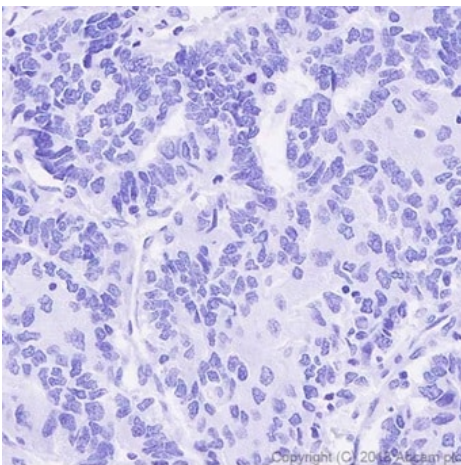


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606)

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 paraffin-embedded 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.

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

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

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

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