abcam

Product datasheet

Anti-PUS1 antibody [EPR20181] ab203010

敲除验证 重组 RabMAb

1 References 10 图像

概述

产品名称	Anti-PUS1抗体[EPR20181]
描述	兔单克隆抗体[EPR20181] to PUS1
宿主	Rabbit
经测试应用	**适用于:** WB, ICC/IF, IP, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Human skeletal muscle lysate; HeLa mitchodrial and cytoplasm fraction lysates; A431, Daudi, HEK-293, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Mouse heart and spleen lysates; Rat spleen lysate. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HEK-293 whole cell lysate.
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**.

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR20181

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab203010**于以下的**经测试应用

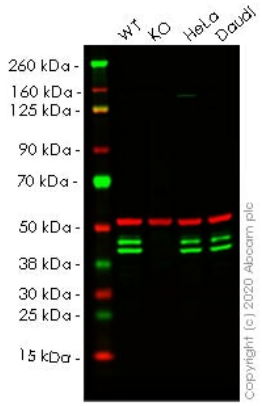
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		1/1000. Detects a band of approximately 47, 44 kDa (predicted molecular weight: 47 kDa).
ICC/IF		1/500. This antibody was not successful when we used it on RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells in ICC application. This antibody was not tested on rat cells in ICC.
IP		1/30.
Flow Cyt (Intra)		1/400.

靶标

功能	Converts specific uridines to PSI in a number of tRNA substrates. Acts on positions 27/28 in the anticodon stem and also positions 34 and 36 in the anticodon of an intron containing tRNA. Involved in regulation of nuclear receptor activity possibly through pseudouridylation of SRA1 RNA.
组织特异性	Widely expressed. High levels of expression found in brain and skeletal muscle.
疾病相关	Defects in PUS1 are a cause of myopathy with lactic acidosis and sideroblastic anemia type 1 (MLASA1) [MIM:600462]; also known as mitochondrial myopathy and sideroblastic anemia. MLASA is a rare autosomal recessive oxidative phosphorylation disorder specific to skeletal muscle and bone marrow.
序列相似性	Belongs to the tRNA pseudouridine synthase TruA family.
细胞定位	Mitochondrion and Nucleus.

图片



Western blot - Anti-PUS1 antibody [EPR20181] (ab203010)

All lanes : Anti-PUS1 antibody [EPR20181] (ab203010) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : PUS1 knockout HEK-293T cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Daudi cell lysate

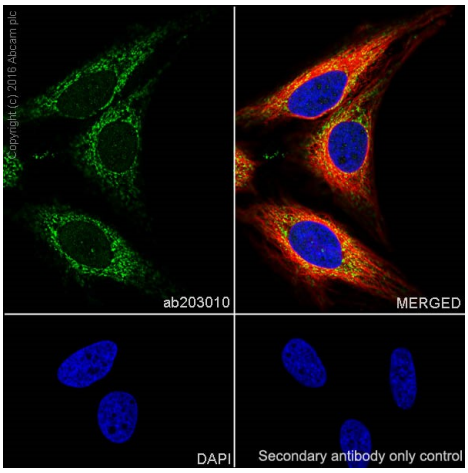
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 47 kDa
Observed band size: 45 kDa

Lanes 1- 4: Merged signal (red and green). Green - ab203010 observed at 45 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab203010 was shown to react with PUS1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab266091** (knockout cell lysate **ab258158**) was used. Wild-type HEK-293T and PUS1 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab203010 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



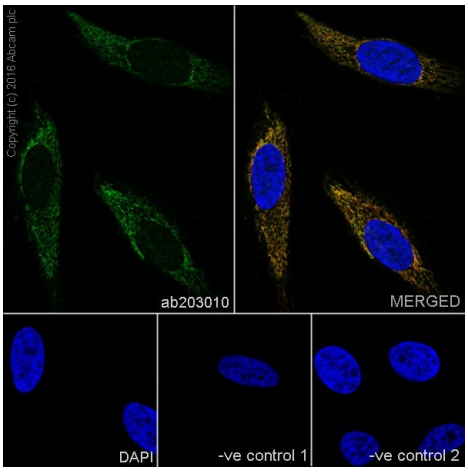
Immunocytochemistry/ Immunofluorescence - Anti-PUS1 antibody [EPR20181] (ab203010)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling PUS1 with ab203010 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cell line.

The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

This antibody was not successful when we used it on RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells in ICC application. This antibody was not tested on rat cells in ICC.



Immunocytochemistry/ Immunofluorescence - Anti-PUS1 antibody [EPR20181] (ab203010)

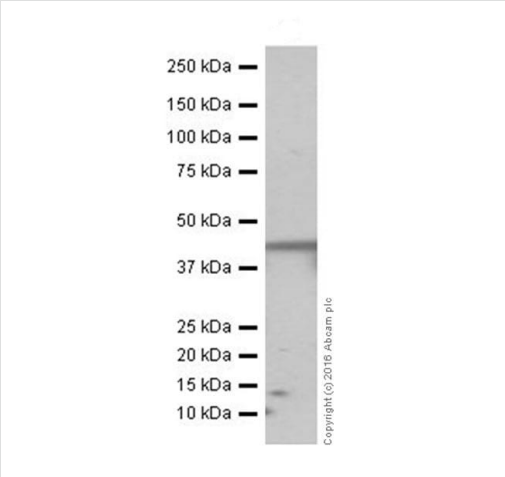
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling PUS1 with ab203010 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing mitochondrial staining on HeLa cell line.

The nuclear counterstain is DAPI (blue). COXIV is detected with **ab33985** (Anti-COX IV(mouse mAb)) at 1/1000 dilution followed by Goat anti-mouse IgG (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab203010 at 1/500 dilution followed by **ab150120** (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/1000 dilution.

-ve control 2: **ab33985** (anti-COX IV(mouse mAb)) at 1/1000 dilution followed by **ab150077** (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/1000 dilution.



Western blot - Anti-PUS1 antibody [EPR20181] (ab203010)

Anti-PUS1 antibody [EPR20181] (ab203010) at 1/1000 dilution + Human skeletal muscle lysate at 10 µg

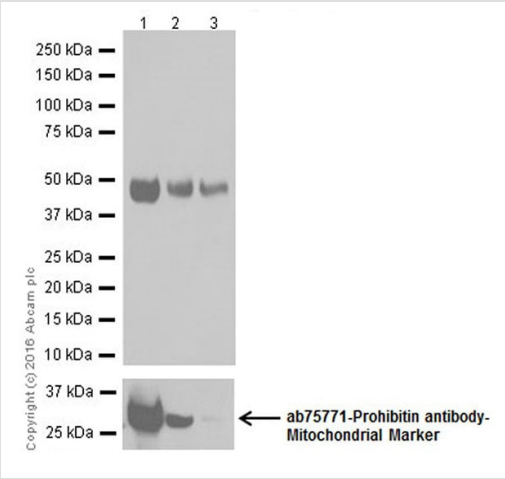
Secondary

Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 47 kDa
Observed band size: 47 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-PUS1 antibody [EPR20181] (ab203010)

All lanes : Anti-PUS1 antibody [EPR20181] (ab203010) at 1/5000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) mitochondria lysate
Lane 2 : HeLa cytoplasm fraction lysate
Lane 3 : A431 (Human epidermoid carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

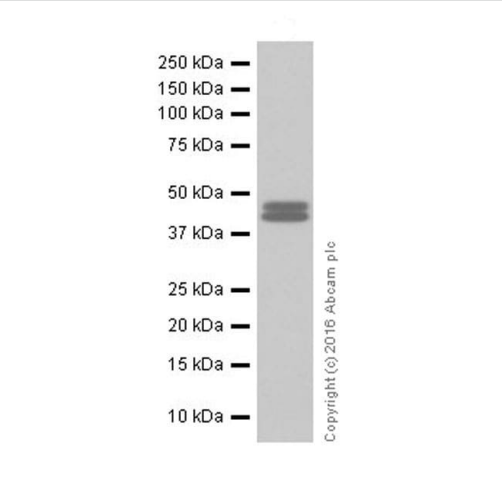
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 47 kDa
Observed band size: 47 kDa

Exposure time: 8 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in UniProt.



Western blot - Anti-PUS1 antibody [EPR20181] (ab203010)

Anti-PUS1 antibody [EPR20181] (ab203010) at 1/1000 dilution + HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 10 µg

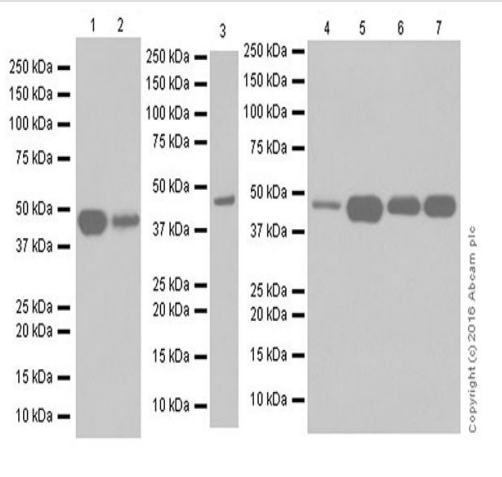
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 47 kDa
Observed band size: 44,47 kDa

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Based on sequence alignment, the antibody can recognize 2 isoforms, the predicted MW are 47kDa and 44kDa, respectively [PMID: 17056637].



Western blot - Anti-PUS1 antibody [EPR20181] (ab203010)

All lanes : Anti-PUS1 antibody [EPR20181] (ab203010) at 1/2000 dilution

Lane 1 : Mouse heart tissue lysate
Lane 2 : Mouse spleen tissue lysate
Lane 3 : Rat spleen tissue lysate
Lane 4 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 5 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 6 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 7 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

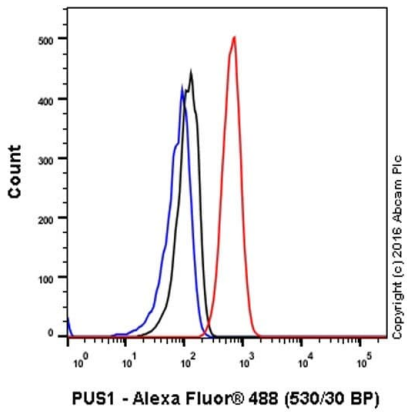
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 47 kDa

Observed band size: 47 kDa

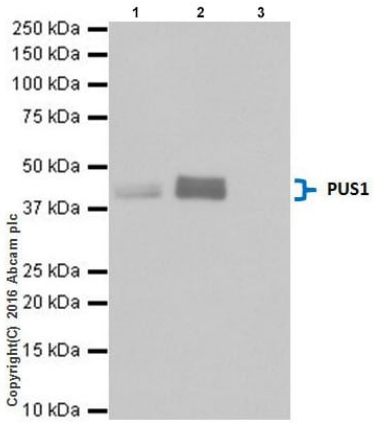
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 10 seconds; Lane 3: 3minutes; Lane 4,5,6 and 7: 10 seconds.



Flow Cytometry (Intracellular) - Anti-PUS1 antibody [EPR20181] (ab203010)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling PUS1 with ab203010 at 1/400 dilution (red) compared with a 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) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-PUS1 antibody [EPR20181] (ab203010)

PUS1 was immunoprecipitated from 0.35 mg of HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate with ab203010 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab203010 at 1/1000 dilution. VeriBlot for IP Detection Reaction (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

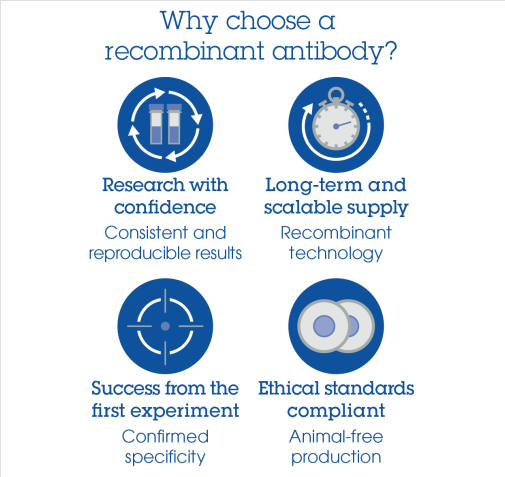
Lane 1: HEK-293 whole cell lysate, 10µg (Input).

Lane 2: ab203010 IP in HEK-293 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab203010 in HEK-293 whole cell lysate.

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

Exposure time: 5 seconds.



Anti-PUS1 antibody [EPR20181] (ab203010)

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