abcam

Product datasheet

Anti-DUSP4 antibody [EPR19881] ab216576

敲除验证 重组 RabMAb

10 References 11 图像

概述

产品名称	Anti-DUSP4抗体[EPR19881]
描述	兔单克隆抗体[EPR19881] to DUSP4
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, IP, WB, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: MDA-MB-231, A549, Wild-type A549, SK-BR-3, HCT 116, RAW 264.7, PC-12, MOLT-4 and C6 whole cell lysates; Human breast cancer lysate. ICC/IF: A549 and MDA-MB-231 cells. Flow Cyt (intra): MDA-MB-231 cells, A549 cells. IP: MDA-MB-231 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, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR19881

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab216576于以下的经测试应用**

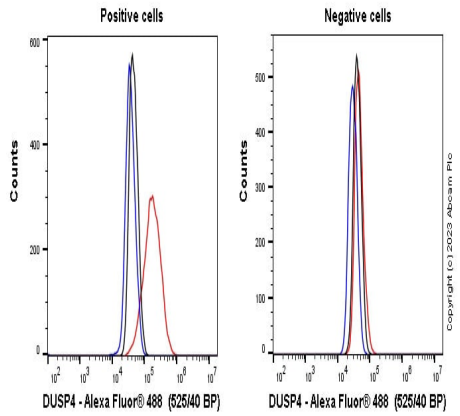
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		Use a concentration of 1 µg/ml. This product gave a positive signal in A549 (DUSP4 knockout A549 cells used as a negative control) fixed with 100% methanol (5 min).
IP		1/30.
WB		1/1000. Detects a band of approximately 43 kDa (predicted molecular weight: 43 kDa).
Flow Cyt (Intra)		1/60. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

靶标

功能	Regulates mitogenic signal transduction by dephosphorylating both Thr and Tyr residues on MAP kinases ERK1 and ERK2.
序列相似性	Belongs to the protein-tyrosine phosphatase family. Non-receptor class dual specificity subfamily. Contains 1 rhodanese domain. Contains 1 tyrosine-protein phosphatase domain.
翻译后修饰	Phosphorylation in the C-terminus by ERK1/2 inhibits proteasomal degradation and stabilizes the protein.
细胞定位	Nucleus.

图片



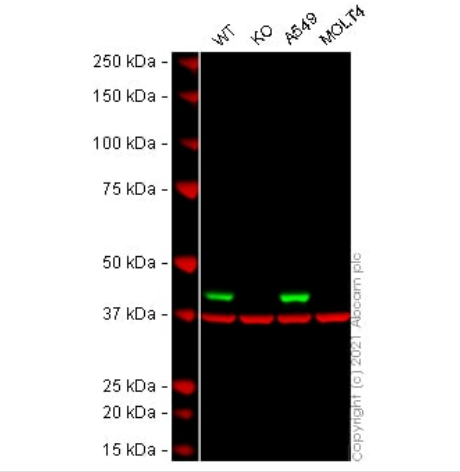
Flow Cytometry (Intracellular) - Anti-DUSP4 antibody [EPR19881] (ab216576)

Flow cytometry overlay histogram showing left wild-type A549 positive cells and right negative DUSP4 knockout A549 stained with ab216576 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab216576) (1x 10^6 in 100µl at 1.0 µg/ml (1/1990)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) was used at the same concentration and conditions as the primary antibody. Unlabelled sample was also used as a control (blue line).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-DUSP4 antibody [EPR19881] (ab216576)

All lanes : Anti-DUSP4 antibody [EPR19881] (ab216576) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : DUSP4 knockout A549 cell lysate
Lane 3 : A549 cell lysate
Lane 4 : MOLT-4 cell lysate

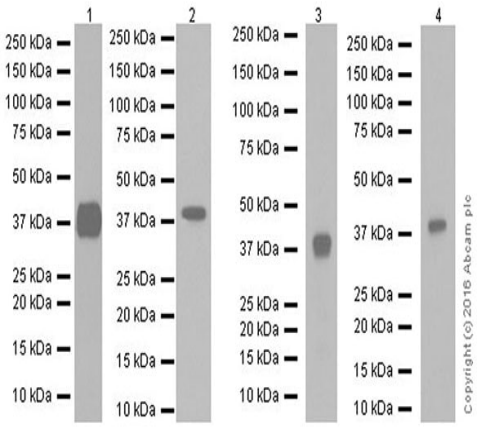
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 43 kDa
Observed band size: 40 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab216576 observed at 40 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab216576 was shown to react with DUSP4 in wild-type A549 cells in Western blot with loss of signal observed in DUSP4 knockout cell line **ab273859** (DUSP4 knockout cell lysate **ab273813**). Wild-type A549 and DUSP4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab216576 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-DUSP4 antibody [EPR19881] (ab216576)

All lanes : Anti-DUSP4 antibody [EPR19881] (ab216576) at 1/1000 dilution

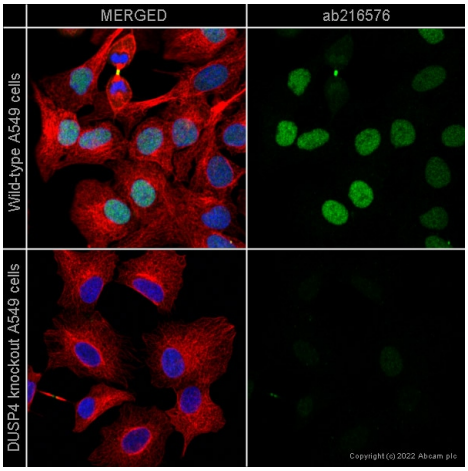
Lane 1 : MDA-MB-231 (Human breast adenocarcinoma cell line) whole cell lysate at 20 µg
Lane 2 : A549 (Human lung carcinoma cell line) whole cell lysate at 10 µg
Lane 3 : SK-BR-3 (Human mammary gland adenocarcinoma cell line) whole cell lysate at 10 µg
Lane 4 : HCT 116 (Human colorectal carcinoma cell line) whole cell lysate at 10 µg

Secondary
Lanes 1-2 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution
Lanes 3-4 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 43 kDa
Observed band size: 43 kDa

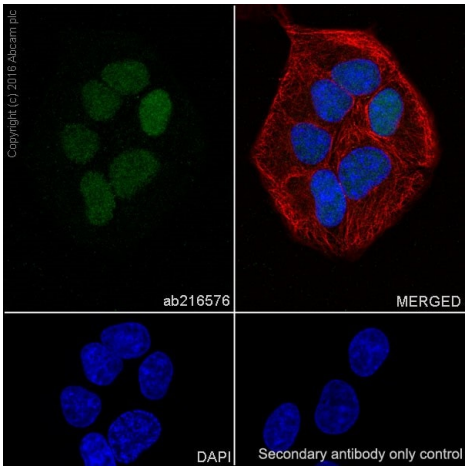
Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2:3 minutes; Lane 3: 30 seconds; Lane 4: 1 second.



Immunocytochemistry/ Immunofluorescence - Anti-DUSP4 antibody [EPR19881] (ab216576)

ab216576 staining DUSP4 in wild-type A549 cells, with negative expression in DUSP4 knockout A549 cells. The cells were fixed with 100% methanol (5 min), permeabilised 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 ab216576 at 1 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150119**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 647), pre-adsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



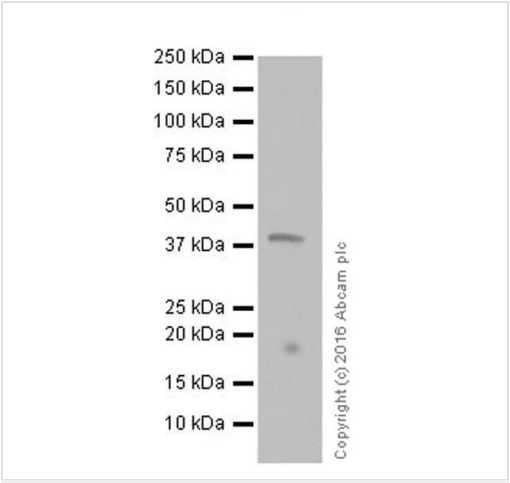
Immunocytochemistry/ Immunofluorescence - Anti-DUSP4 antibody [EPR19881] (ab216576)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (Human lung carcinoma cell line) cells labeling DUSP4 with ab216576 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on A549 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.



Western blot - Anti-DUSP4 antibody [EPR19881] (ab216576)

Anti-DUSP4 antibody [EPR19881] (ab216576) at 1/1000 dilution + Human breast cancer lysate at 10 µg

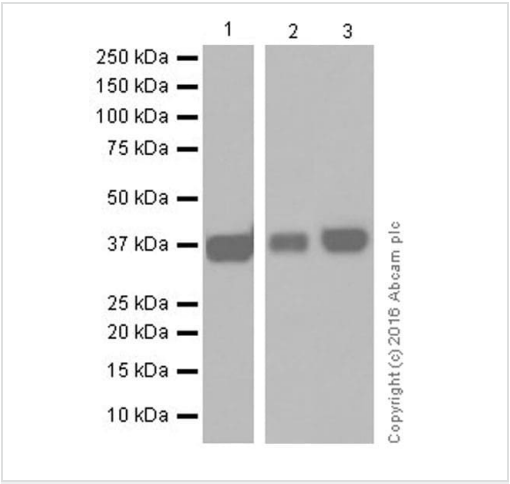
Secondary

VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/1000 dilution

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-DUSP4 antibody [EPR19881] (ab216576)

All lanes : Anti-DUSP4 antibody [EPR19881] (ab216576) at 1/1000 dilution

Lane 1 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 3 : C6 (Rat glial tumor cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

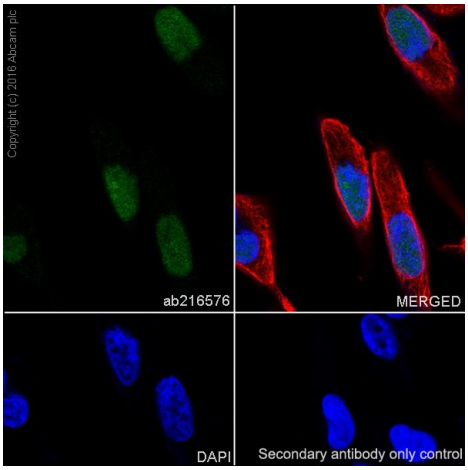
Secondary

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

Predicted band size: 43 kDa
Observed band size: 43 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1:3 minutes; Lane 2/3: 15 seconds.



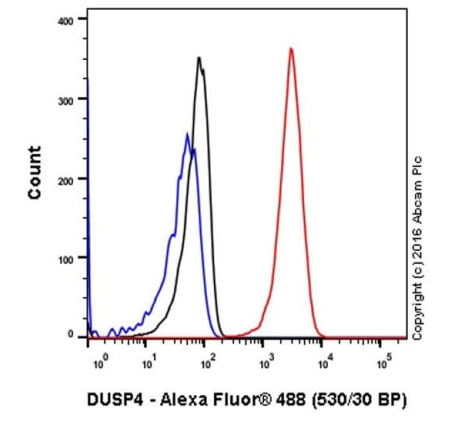
Immunocytochemistry/ Immunofluorescence - Anti-DUSP4 antibody [EPR19881] (ab216576)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MDA-MB-231 (Human breast adenocarcinoma cell line) cells labeling DUSP4 with ab216576 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on MDA-MB-231 cell line.

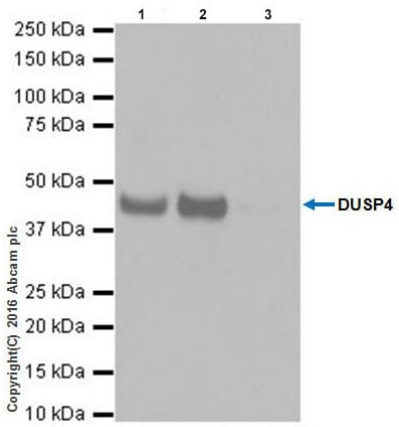
The nuclear counterstain is DAPI (blue). Tubulin is detected with **ab195889** (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

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.



Flow Cytometry (Intracellular) - Anti-DUSP4 antibody [EPR19881] (ab216576)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed MDA-MB-231 (Human breast adenocarcinoma cell line) cells labeling DUSP4 with ab216576 at 1/60 dilution (red) compared with a rabbit monoclonal IgG isotype control (**ab172730**; black) and an unlabelled 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-DUSP4 antibody [EPR19881] (ab216576)

DUSP4 was immunoprecipitated from 0.35mg of MDA-MB-231 (Human breast adenocarcinoma cell line) whole cell lysate with ab216576 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab216576 at 1/500 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1,000 dilution

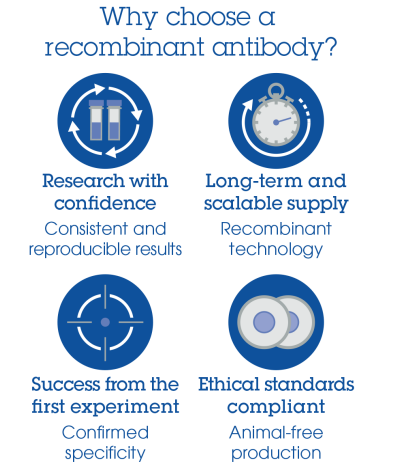
Lane 1: MDA-MB-231 whole cell lysate, 10µg (Input).

Lane 2: ab216576 IP in MDA-MB-231 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab216576 in MDA-MB-231 whole cell lysate.

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

Exposure time: 1 second.



Anti-DUSP4 antibody [EPR19881] (ab216576)

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