

Anti-DUSP4 antibody [EPR19881] - BSA and Azide free ab222487

KO VALIDATED

Recombinant

RabMAb

8 Images

Overview

Product name	Anti-DUSP4 antibody [EPR19881] - BSA and Azide free
Description	Rabbit monoclonal [EPR19881] to DUSP4 - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	<p>ab222487 is the carrier-free version of ab216576.</p> <p>Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.</p> <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</p>

monoclonal antibodies. For details on our patents, please refer to [**RabMAb® patents**](#).

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19881
Isotype	IgG

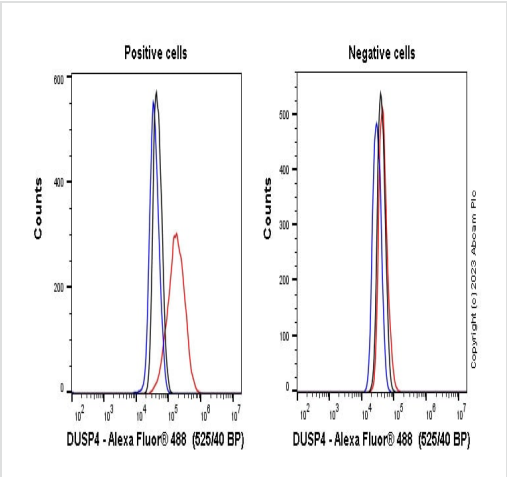
Applications

The Abpromise guarantee Our [**Abpromise guarantee**](#) covers the use of ab222487 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration. This product gave a positive signal in A549 (DUSP4 knockout A549 cells used as a negative control) fixed with 100% methanol (5 min).
WB		Use at an assay dependent concentration. Detects a band of approximately 43 kDa (predicted molecular weight: 43 kDa).
IP		Use at an assay dependent concentration.

Target

Function	Regulates mitogenic signal transduction by dephosphorylating both Thr and Tyr residues on MAP kinases ERK1 and ERK2.
Sequence similarities	Belongs to the protein-tyrosine phosphatase family. Non-receptor class dual specificity subfamily. Contains 1 rhodanese domain. Contains 1 tyrosine-protein phosphatase domain.
Post-translational modifications	Phosphorylation in the C-terminus by ERK1/2 inhibits proteasomal degradation and stabilizes the protein.
Cellular localization	Nucleus.



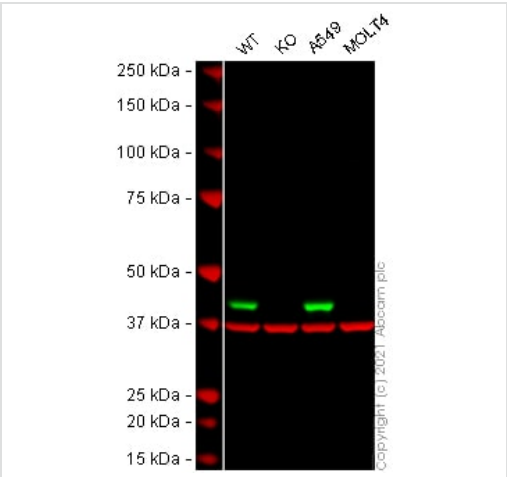
Flow Cytometry (Intracellular) - Anti-DUSP4 antibody [EPR19881] - BSA and Azide free (ab222487)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([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 10⁶ 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] - BSA and Azide free (ab222487)

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.

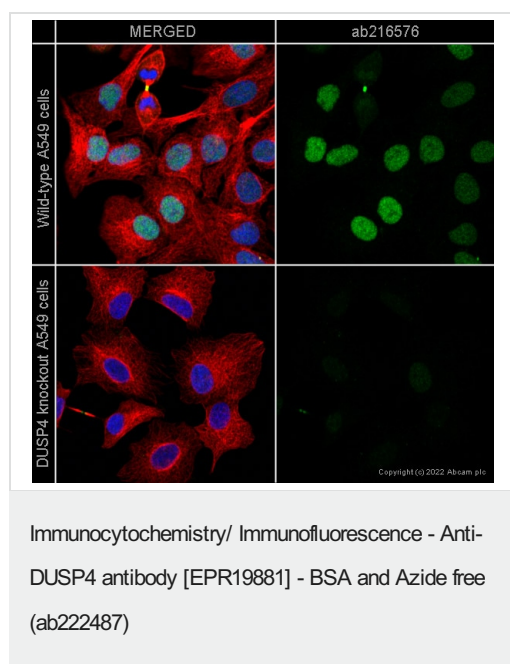
Predicted band size: 43 kDa
Observed band size: 40 kDa

This data was developed using the same antibody clone in a different buffer formulation ([ab216576](#)).

Lanes 1 -4: Merged signal (red and green). Green - [ab216576](#) observed at 40 kDa. Red - loading control [ab8245](#) (Mouse anti-

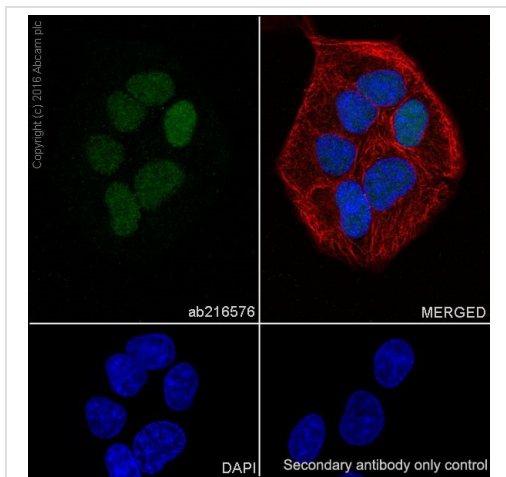
GAPDH antibody [6C5]) observed at 37 kDa.

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.



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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216576**).



Immunocytochemistry/ Immunofluorescence - Anti-DUSP4 antibody [EPR19881] - BSA and Azide free (ab222487)

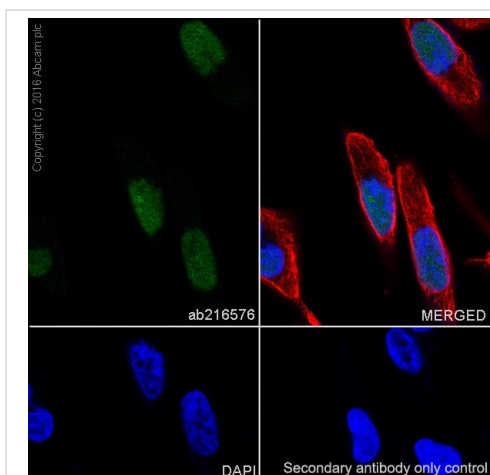
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (Human lung carcinoma cell line) cells labeling DUSP4 with **ab216576** at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216576**).



Immunocytochemistry/ Immunofluorescence - Anti-DUSP4 antibody [EPR19881] - BSA and Azide free (ab222487)

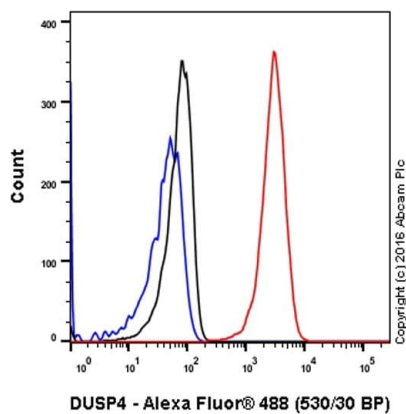
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MDA-MB-231 (Human breast adenocarcinoma cell line) cells labeling DUSP4 with **ab216576** at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution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

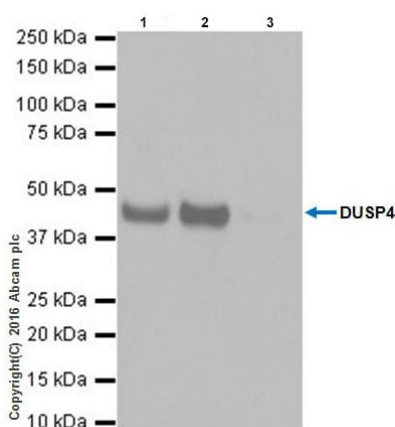
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216576**).



Flow Cytometry (Intracellular) - Anti-DUSP4 antibody
[EPR19881] - BSA and Azide free (ab222487)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216576**).



Immunoprecipitation - Anti-DUSP4 antibody
[EPR19881] - BSA and Azide free (ab222487)

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 1/1000 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab216576**).

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-DUSP4 antibody [EPR19881] - BSA and Azide free (ab222487)

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

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