# abcam

## Product datasheet

## Anti-DUSP6 antibody [EPR129Y] ab76310



ייבער RabMAb

★★★★★ 16 Abreviews 52 References 画像数 13

#### 製品の概要

製品名 Anti-DUSP6 antibody [EPR129Y]

製品の詳細 Rabbit monoclonal [EPR129Y] to DUSP6

由来種 Rabbit

アプリケーション 適用あり: IHC-P, Flow Cyt (Intra), WB, IP

適用なし: ICC/IF

種交差性 交差種: Mouse, Rat, Human

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

ポジティブ・コントロール WB: 3T3, and A431 cell lysates; IHC-P: Human gastric carcinoma and human pancreas tissues;

IHC-Fr: Mouse brain tissue; Flow Cyt (intra): NIH-3T3 and HeLa cells; IP: NIH-3T3 cells.

特記事項 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**.

#### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 EPR129Y

## アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab76310の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション         | Abreviews        | 特記事項   |
|------------------|------------------|--|
| IHC-P            | <b>★★★★☆</b> (1) | 1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.            |
| Flow Cyt (Intra) |                  | 1/20 - 1/200.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. |
| WB               | <b>★★★★★ (8)</b> | 1/500 - 1/1000. Detects a band of approximately 42,44 kDa (predicted molecular weight: 42 kDa).                |
| IP               | <b>★★★★★</b> (2) | 1/40 - 1/50.   |

追加情報 Is unsuitable for ICC/IF.

## ターゲット情報

機能 Inactivates MAP kinases. Has a specificity for the ERK family.

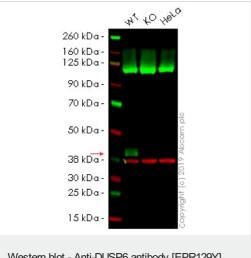
**配列類似性** Belongs to the protein-tyrosine phosphatase family. Non-receptor class dual specificity subfamily.

Contains 1 rhodanese domain.

Contains 1 tyrosine-protein phosphatase domain.

細胞内局在 Cytoplasm.

#### 画像



Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)

**All lanes :** Anti-DUSP6 antibody [EPR129Y] (ab76310) at 1/500 dilution

**Lane 1 :** Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2: DUSP6 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

**Lane 3**: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

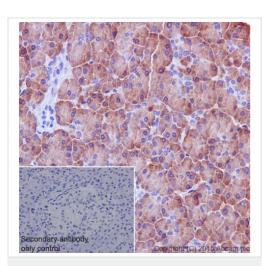
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa

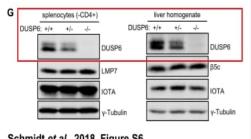
**Lanes 1 - 3:** Merged signal (red and green). Green - ab76310 observed at 42 kDa. Red - loading control, <u>ab130007</u>, observed at 130 kDa.

ab76310 was shown to recognize in wild-type A431 cells as signal was lost at the expected MW in DUSP6 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and DUSP6 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab76310 and <a href="mailto:ab130007">ab130007</a> (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/500 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216773">ab216773</a> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216776">ab216776</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DUSP6 antibody [EPR129Y] (ab76310)

Immunohistochemical staining of paraffin embedded human pancreas with purified ab76310 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perforned using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



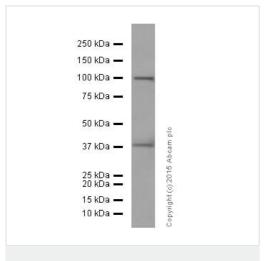
Schmidt et al., 2018, Figure S6

Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)

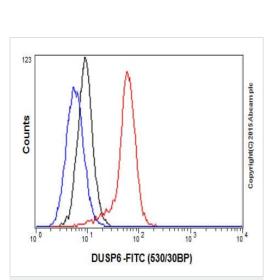
Schmidt C et al., Front Immunol, 9, 2386, 2018 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Immunoblot analysis of indicated proteins in splenocytes after CD4+ sort, liver homogenate or purified CD4+ T cells from WT, DUSP6+/- or DUSP6-/- mice to confirm knockout or heterozygosity for DUSP6 at protein level.

This image was generated using ab220811, the same antibody but in a PBS-only buffer formulation.



Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)



Flow Cytometry (Intracellular) - Anti-DUSP6 antibody [EPR129Y] (ab76310) Anti-DUSP6 antibody [EPR129Y] (ab76310) at 1/5000 dilution (purified) + HepG2 cell lysate at 20 µg

## **Secondary**

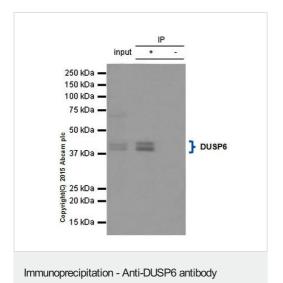
HRP goat anti-rabbit lgG (H+L) at 1/50000 dilution

**Predicted band size:** 42 kDa **Observed band size:** 42,44 kDa

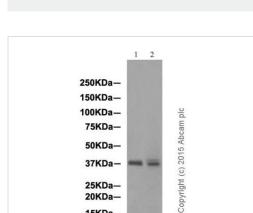
Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

ab76310 detects an unspecific band around 100 kDa in human materials.

Overlay histogram showing NIH-3T3 cells fixed in 4% PFA and stained with purified ab76310 at a dilution of 1 in 200 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).



ab76310 (purified) at 1/20 immunoprecipitating DUSP6 in 10 µg NIH-3T3 (Lanes 1 and 2, observed at 42 and 44 kDa). Lane 3 -PBS. For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



50KDa-37KDa-

25KDa-20KDa-15KDa-

10KDa-

[EPR129Y] (ab76310)

All lanes: Anti-DUSP6 antibody [EPR129Y] (ab76310) at 1/1000 dilution (purified)

Lane 1: rat brain lysate Lane 2: NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

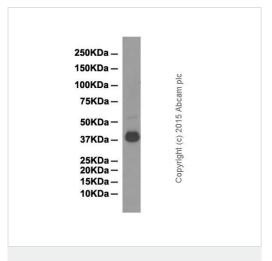
Secondary

All lanes: HRP goat anti-rabbit lgG (H+L) at 1/1000 dilution

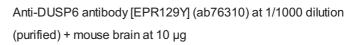
Predicted band size: 42 kDa Observed band size: 42,44 kDa

Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)

> Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)

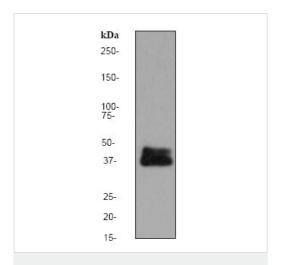


## Secondary

HRP goat anti-rabbit lgG (H+L) at 1/50000 dilution

**Predicted band size:** 42 kDa **Observed band size:** 42,44 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)

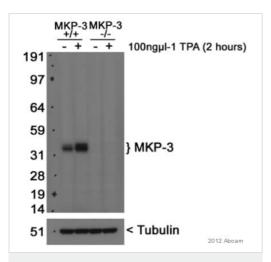
Anti-DUSP6 antibody [EPR129Y] (ab76310) at 1/500 dilution (unpurified) + 3T3 cell lysate at 10  $\mu g$ 

## Secondary

HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 42 kDa

Observed band size: 42.44 kDa



Western blot - Anti-DUSP6 antibody [EPR129Y] (ab76310)

This image was taken from an abreview by Graeme Stewart.

**All lanes :** Anti-DUSP6 antibody [EPR129Y] (ab76310) at 1/4000 dilution (unpurified)

Lane 1: Marker

Lane 2: Lysate from wild type primary murine embryonic fibroblasts (MEFs) untreated at 10 µg

**Lane 3 :** Lysate from wild type primary murine embryonic fibroblasts (MEFs) treated with 100ng $\mu$ l-1, 12-O-tetradecanoylphorbol-13-acetate (TPA) for 2 hours at 10  $\mu$ g

Lane 4: Lysate from MKP-3 null primary murine embryonic fibroblasts (MEFs) untreated at 10 µg

**Lane 5**: Lysate from MKP-3 null primary murine embryonic fibroblasts (MEFs) treated with 100ngμl-1, 12-O-tetradecanoylphorbol-13-acetate (TPA) for 2 hours at 10 μg

### **Secondary**

All lanes: Goat anti Rabbit HRP conjugate at 1/10000 dilution

Developed using the ECL technique.

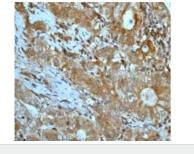
Performed under reducing conditions.

**Predicted band size:** 42 kDa **Observed band size:** 42 kDa

Exposure time: 2 minutes

Immunohistochemical staining of paraffin-embedded human gastric carcinoma using unpurified ab76310 at 1/50 dilution.

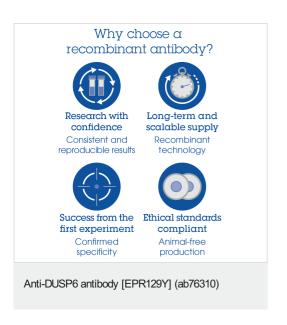
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-DUSP6 antibody
[EPR129Y] (ab76310)

Flow Cytometry (Intracellular) - Anti-DUSP6 antibody [EPR129Y] (ab76310) Overlay histogram showing HeLa cells stained with unpurifiedab76310 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions

followed by the antibody (ab76310, 1/20 dilution) for 30 min at 22°C. The secondary antibody used was DyLight<sup>®</sup> 488 goat antirabbit  $\lg G$  (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal  $\lg G$  (1 $\mu$ g/1x10 $^6$  cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde/permeabilized with 0.1% PBS-Tween 20 used under the same conditions.



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