abcam

Product datasheet

Anti-CD27 antibody [EPR8569] - BSA and Azide free ab256583



ייבע RabMAb

2 References 画像数 11

製品の概要

製品名 Anti-CD27 antibody [EPR8569] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR8569] to CD27 - BSA and Azide free

由来種 Rabbit

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

種交差性 交差種: 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: Raji, Ramos and NAMALWA cell lysates and human lymph node and fetal spleen tissue

lysates. IHC-P: Human stomach and tonsil tissues. ICC/IF: Jurkat cells. Flow Cyt (intra): Ramos

cells. Human PBMCs.

特記事項 ab256583 is the carrier-free version of ab131254.

> 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.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

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.

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.

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. Do Not Freeze.

バッファー pH: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR8569

アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab256583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
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 55 kDa (predicted molecular weight: 29 kDa).
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

ターゲット情報

機能 Receptor for CD70/CD27L. May play a role in survival of activated T-cells. May play a role in

apoptosis through association with SIVA1.

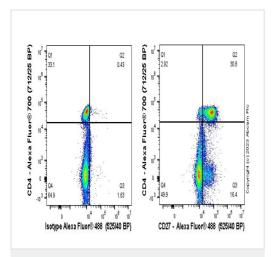
組織特異性 Found in most T-lymphocytes.

配列類似性 Contains 3 TNFR-Cys repeats.

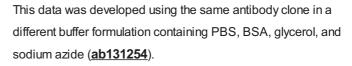
翻訳後修飾 Phosphorylated and O-glycosylated.

細胞内局在 Membrane.

画像



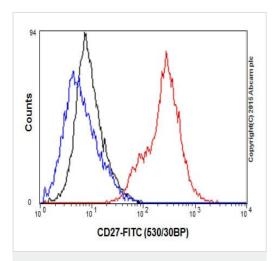
Flow Cytometry (Intracellular) - Anti-CD27 antibody [EPR8569] - BSA and Azide free (ab256583)



Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with <u>ab131254</u> (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Cells were fixed and permeabilised with BD Cytofix/Cytoperm™ for 20 min. PBMCs were incubated for 30 min at 22°C in 1x PBS containing 10 μg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody <u>ab131254</u> or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10⁶ in 100 μl at 0.04 μg/ml (1/52750)) for 30 min at 4°C . The cells were simultaneously stained with CD4.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 4°C

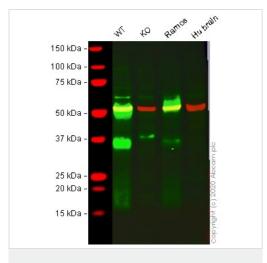
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.



Flow Cytometry (Intracellular) - Anti-CD27 antibody [EPR8569] - BSA and Azide free (ab256583)

Flow Cytometry analysis of Ramos cells labelling CD27 with purified <u>ab131254</u> at 1/300 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal lgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab131254).



Western blot - Anti-CD27 antibody [EPR8569] - BSA and Azide free (ab256583)

All lanes: Anti-CD27 antibody [EPR8569] (ab131254) at 1/1000 dilution

Lane 1 : Wild-type Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 2 : CD27 knockout Raji (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 3 : Ramos (Human Burkitt's lymphoma cell line) whole cell lysate

Lane 4: Human brain tissue lysate

Lysates/proteins at 20 µg per lane.

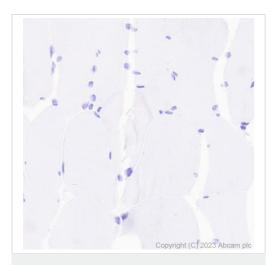
Performed under reducing conditions.

Predicted band size: 29 kDa Observed band size: 35 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab131254</u>).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab131254</u> observed at 35 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab131254 was shown to react with CD27 in wild-type Raji cells in western blot with loss of signal observed in CD27 knockout sample. Wild-type and CD27 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab131254 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) 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 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD27 antibody

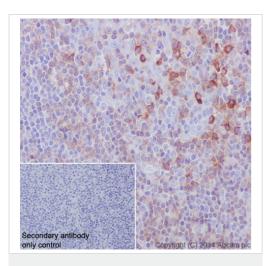
[EPR8569] - BSA and Azide free (ab256583)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab131254).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle showing negative staining with purified <u>ab131254</u> at 1/1800. Heat mediated antigen retrieval was performed using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). <u>ab214880</u>, a Goat Anti-Rabbit IgG H&L (HRP polymer) was used as the secondary antibody (1/500).

Counterstained with hematoxylin.

Negative control: No staining on human skeletal muscle. The section was incubated with <u>ab131254</u> at 4°C overnight.

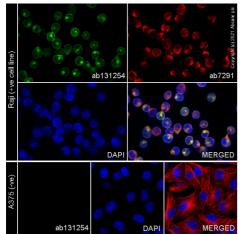


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD27 antibody

[EPR8569] - BSA and Azide free (ab256583)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling CD27 with purified ab131254 at 1/1500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab131254</u>).



Immunocytochemistry/ Immunofluorescence - Anti-CD27 antibody [EPR8569] - BSA and Azide free (ab256583)

MERGE

Immunocytochemistry/ Immunofluorescence - Anti-CD27 antibody [EPR8569] - BSA and Azide free (ab256583)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab131254).

ab131254 staining CD27 in Raji cells, with negative expression in A375 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 ab131254 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.

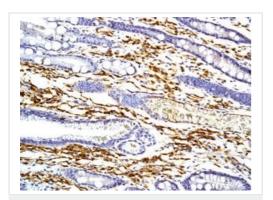
This product also work with 4% formaldehyde (10 min) fixation under the same testing conditions.

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling CD27 with purified ab131254 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat antimouse IgG (1/500) were also used.

Control 1: primary antibody (1/500) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor® 488-conjugated goat anti-rabbit lgG (1/500).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab131254).

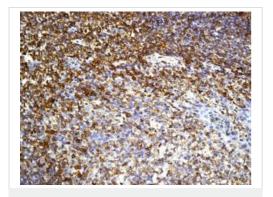


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD27 antibody

[EPR8569] - BSA and Azide free (ab256583)

Immunohistochemistry (Formalin/PFA-fixed paraffin embedded sections) analysis of human stomach tissue labelling CD27 with **ab131254** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab131254</u>).

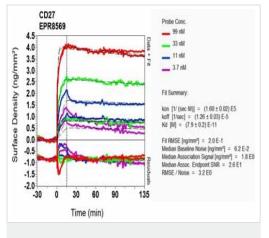


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD27 antibody

[EPR8569] - BSA and Azide free (ab256583)

Immunohistochemistry (Formalin/PFA-fixed paraffin embedded sections) analysis of human tonsil tissue labelling CD27 with **ab131254** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab131254).



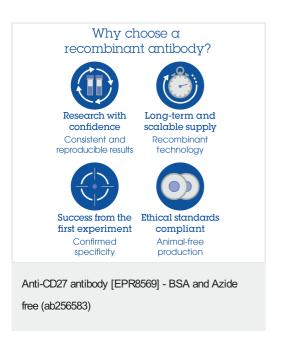
Ol-RD Scanning - Anti-CD27 antibody [EPR8569] - BSA and Azide free (ab256583)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about KD

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab131254</u>).



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