

Anti-CD27 antibody [EPR8569] ab131254

敲除验证 重组 RabMAb

★★★★★ 2 Abreviews 16 References 14 图像

概述

产品名称	Anti-CD27抗体[EPR8569]
描述	兔单克隆抗体[EPR8569] to CD27
宿主	Rabbit
经测试应用	适用于: ICC/IF, WB, IHC-P, Flow Cyt (Intra)
种属反应性	与反应: Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab192535)
阳性对照	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: Ramos cells, Human PBMCs.
常规说明	<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 monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

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. Stable for 12 months at -20°C.
解离常数 (K _D)	K _D = 7.90 x 10 ⁻¹¹ M





[Learn more about K_D](#)

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR8569
同种型	IgG

应用

The Abpromise guarantee [Abpromise™](#)承诺保证使用ab131254于以下的经测试应用

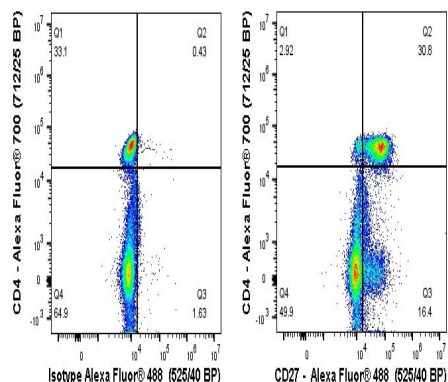
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		1/250 - 1/500.
WB		1/1000. Detects a band of approximately 55 kDa (predicted molecular weight: 29 kDa).Can be blocked with CD27 peptide (ab192535) . For unpurified use at 1/1000 - 1/10000.
IHC-P	★★★★★ (2)	1/1500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols . For unpurified use at 1/100 - 1/250.
Flow Cyt (Intra)		1/100 - 1/500. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

靶标

功能	Receptor for CD70/CD27L. May play a role in survival of activated T-cells. May play a role in apoptosis through association with SIVA1.
组织特异性	Found in most T-lymphocytes.
序列相似性	Contains 3 TNFR-Cys repeats.
翻译后修饰	Phosphorylated and O-glycosylated.
细胞定位	Membrane.

图片

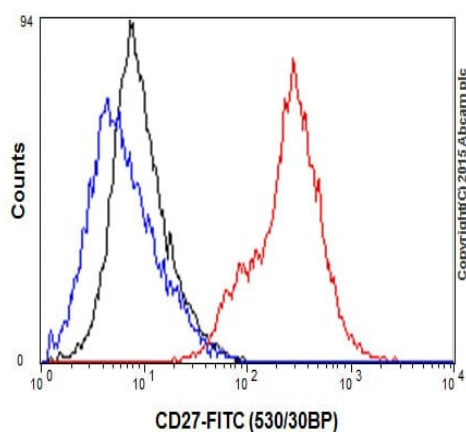


Flow Cytometry (Intracellular) - Anti-CD27 antibody
[EPR8569] (ab131254)

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab131254 (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 ab131254 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1×10^6 in 100 µl at 0.04 µg/ml (1/52750)) for 30 min at 4°C. The cells were simultaneously stained with CD4.

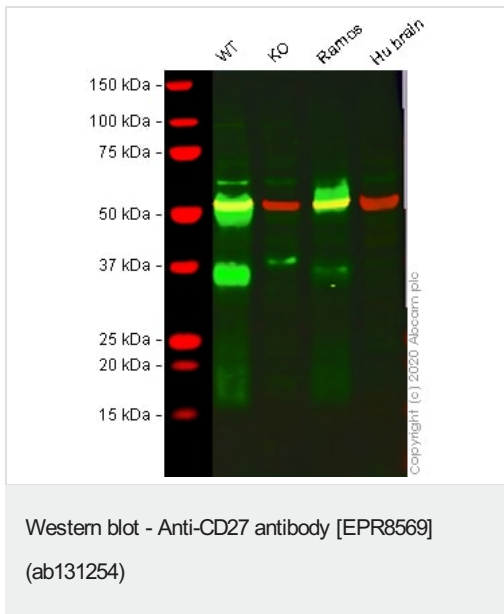
The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 4°C

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] (ab131254)

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



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.

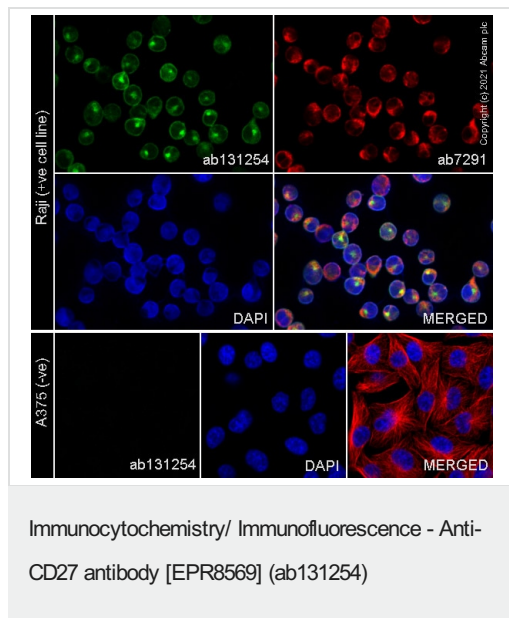
Performed under reducing conditions.

Predicted band size: 29 kDa

Observed band size: 35 kDa

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

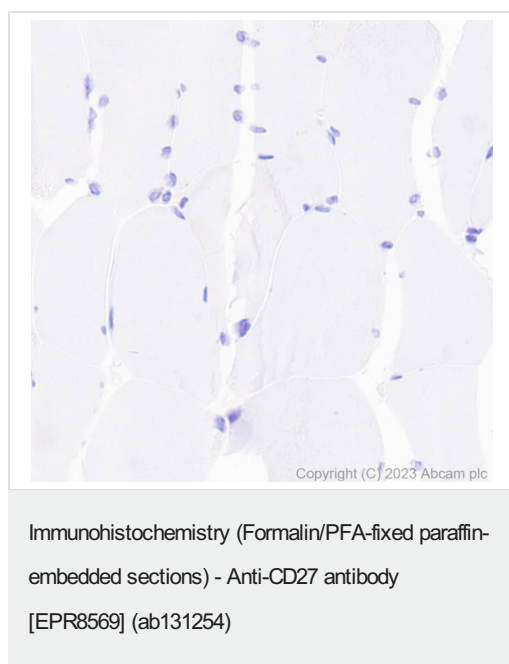
ab131254 was shown to react with CD27 in wild-type Raji cells in western blot with loss of signal observed in CD27 knockout sample. Wild-type and CD27 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween[®]) before incubation with ab131254 and **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.



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.

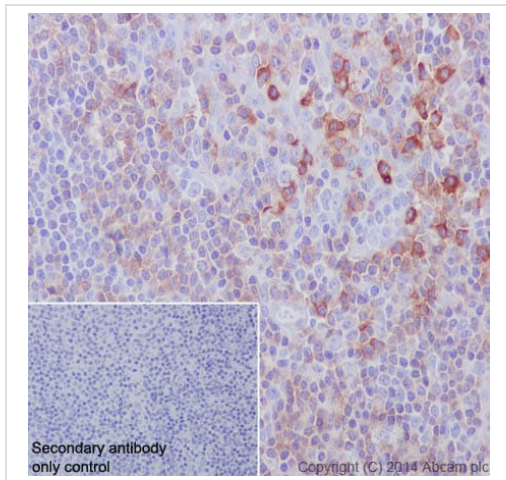
This product also work with 4% formaldehyde (10 min) fixation under the same testing conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle showing negative staining with purified ab131254 at 1/1800. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). **ab214880**, 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 ab131254 at 4°C overnight.

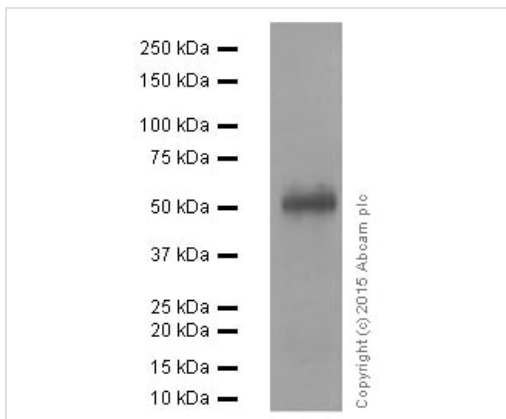


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] (ab131254)

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.



Western blot - Anti-CD27 antibody [EPR8569] (ab131254)

Anti-CD27 antibody [EPR8569] (ab131254) at 1/5000 dilution (purified) + NAMALWA (human Burkitt's lymphoma cell line) cell lysate at 20 µg

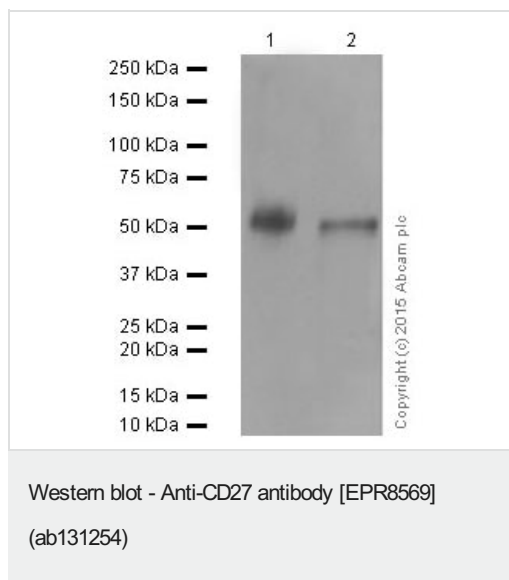
Secondary

Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 29 kDa

Observed band size: 55 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



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

Lane 1 : Human lymph node tissue lysate

Lane 2 : Human fetal spleen tissue lysate

Lysates/proteins at 20 µg per lane.

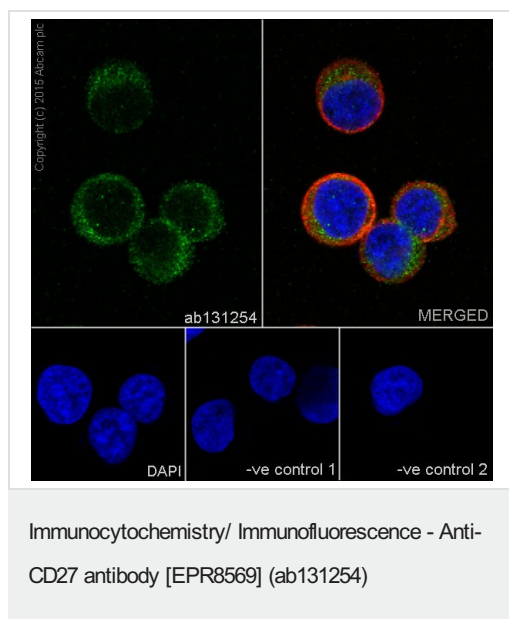
Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 29 kDa

Observed band size: 55 kDa

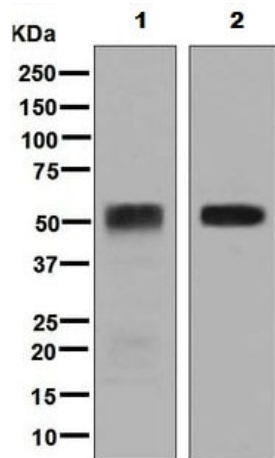
Blocking and dilution buffer: 5% NFDM/TBST.



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 anti-mouse 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: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500).



Western blot - Anti-CD27 antibody [EPR8569] (ab131254)

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

Lane 1 : Ramos (human Burkitt's lymphoma cell line) cell lysate

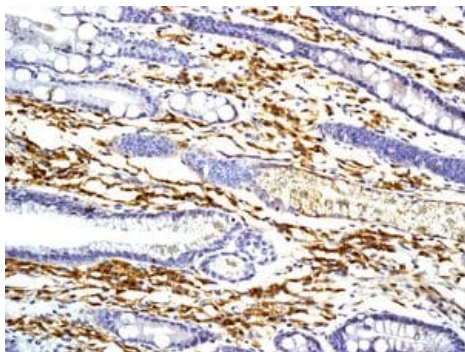
Lane 2 : Human lymph node cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP labelled Goat anti Rabbit IgG at 1/2000 dilution

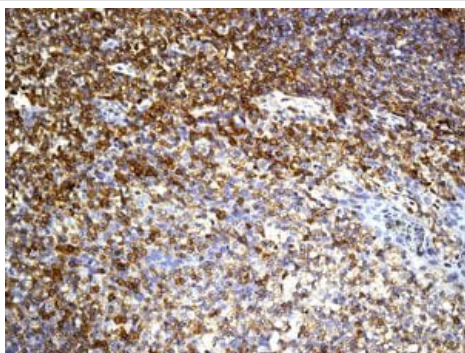
Predicted band size: 29 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] (ab131254)

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

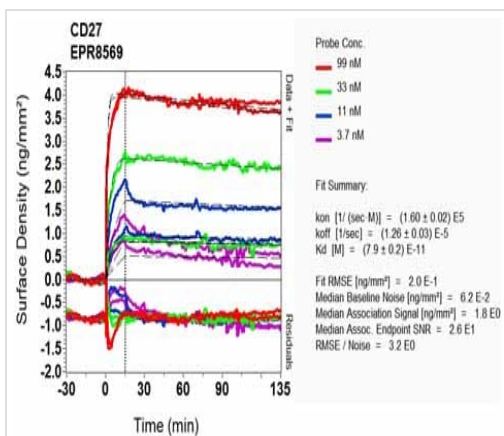
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] (ab131254)

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

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



SPR Scanning - Anti-CD27 antibody [EPR8569]
(ab131254)

Equilibrium dissociation constant (K_D)

Learn more about K_D

[Click here to learn more about \$K_D\$](#)

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Anti-CD27 antibody [EPR8569] (ab131254)

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