abcam

Product datasheet

Anti-CD27 antibody [EPR8569] ab131254

KO 評価済　リコンビナント　RabMAb

★★★★★ **2 Abreviews**　**16 References**　画像数 14

製品の概要

製品名	Anti-CD27 antibody [EPR8569]
製品の詳細	Rabbit monoclonal [EPR8569] to CD27
由来種	Rabbit
アプリケーション	**適用あり:** ICC/IF, WB, IHC-P, Flow Cyt (Intra)
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab192535**)
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: Ramos cells, Human PBMCs.
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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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. Stable for 12 months at -20°C.
解離定数（K_D値）	K_D = 7.90 x 10^{-11} M

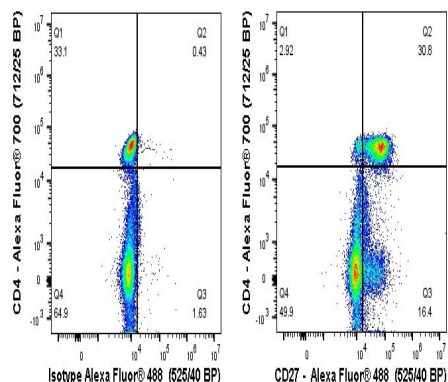|	
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR8569
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee　　　**Abpromise保証は、**次のテスト済みアプリケーションにおけるab131254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
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.

ターゲット情報

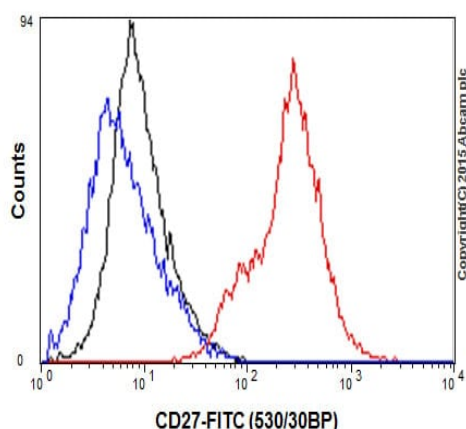|	
機能	Receptor for CD70/CD27L. May play a role in survival of activated T-cells. May play a role in apoptosis through association with SIVA1.
組織特異性	Found in most T-lymphocytes.
配列類似性	Contains 3 TNFR-Cys repeats.
翻訳後修飾	Phosphorylated and O-glycosylated.
細胞内局在	Membrane.

画像

Flow Cytometry (Intracellular) - Anti-CD27 antibody [EPR8569] (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 (1x 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.
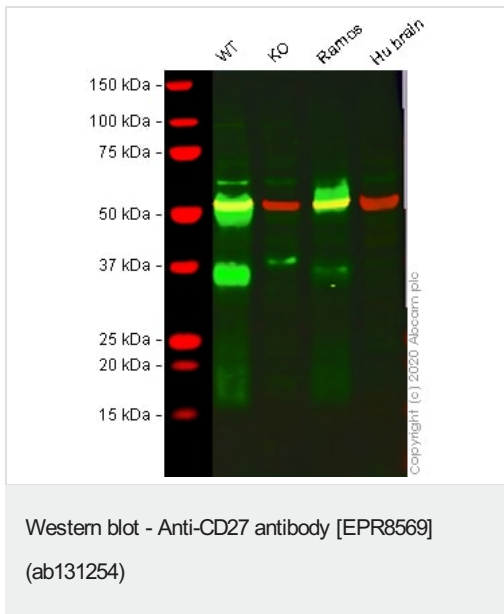
The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 4°C

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.

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

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
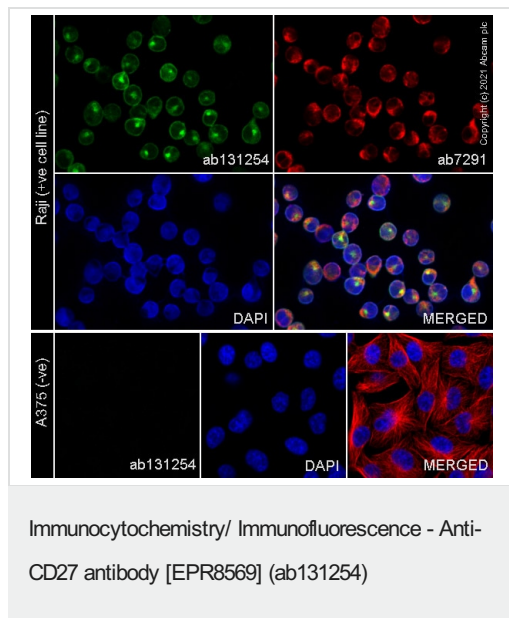
Lysates/proteins at 20 µg per lane.

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.
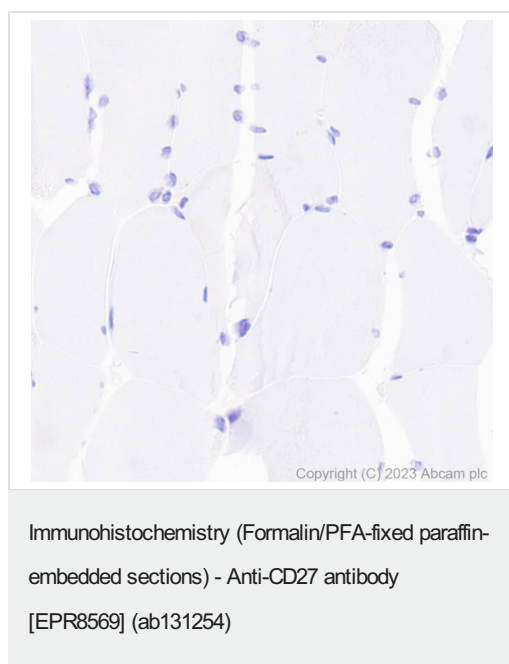
ab131254 was shown to react with CD27 in wild-type Raji cells in western blot with loss of signal observed in CD27 knockout sample. Wild-type and CD27 knockout Raji cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab131254 and **ab7291** (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.

Immunocytochemistry/ Immunofluorescence - Anti-CD27 antibody [EPR8569] (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.
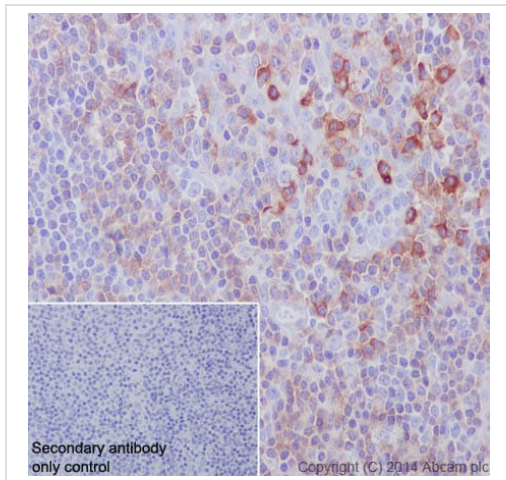
This product also work with 4% formaldehyde (10 min) fixation under the same testing conditions.
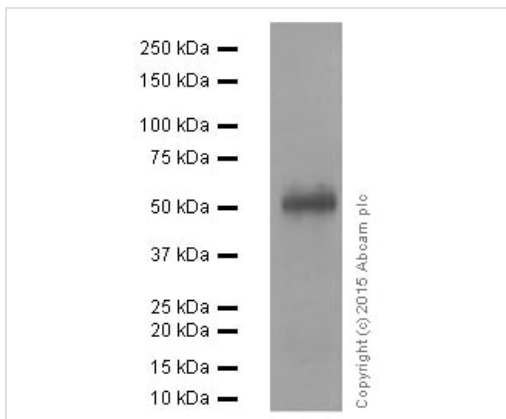

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] (ab131254)

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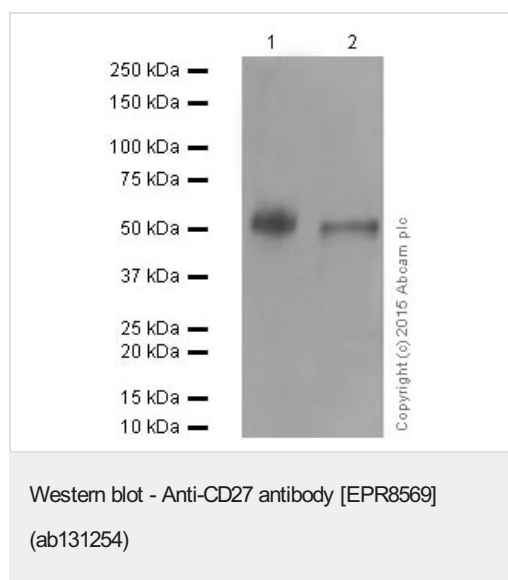
Secondary
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 29 kDa
Observed band size: 55 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

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

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
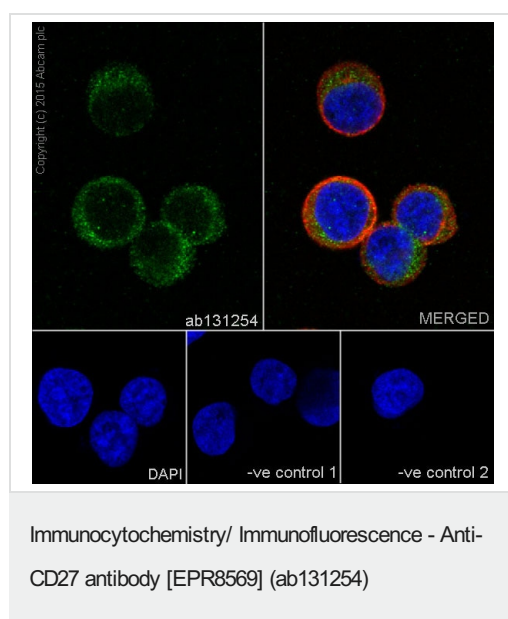
Lysates/proteins at 20 µg per lane.

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 - Anti-CD27 antibody [EPR8569] (ab131254)
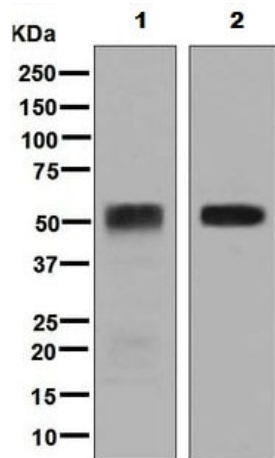
Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling CD27 with purified ab131254 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. 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)

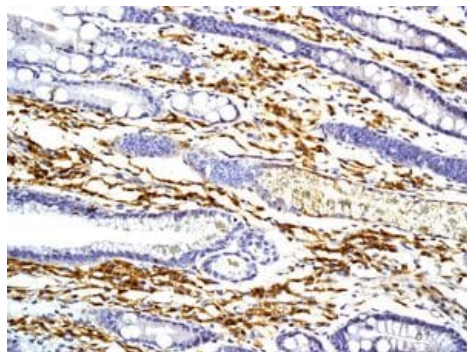**Lane 1 :** Ramos (human Burkitt's lymphoma cell line) cell lysate

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
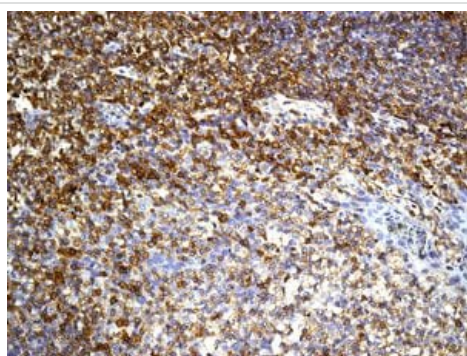
Predicted band size: 29 kDa

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.
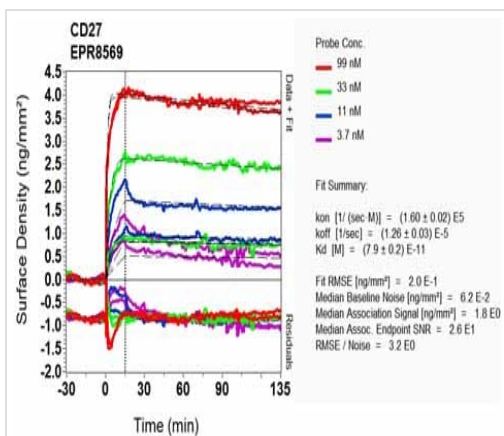

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] (ab131254)

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.

Fit Summary:

kon [1/ (sec·M)] = (1.60 ± 0.02) E5
koff [1/sec] = (1.26 ± 0.03) E-5
Kd [M] = (7.9 ± 0.2) E-11

Fit RMSE [ng/mm²] = 2.0 E-1
Median Baseline Noise [ng/mm²] = 6.2 E-2
Median Association Signal [ng/mm²] = 1.8 E0
Median Assoc. Endpoint SNR = 2.6 E1
RMSE / Noise = 3.2 E0

OI-RD Scanning - Anti-CD27 antibody [EPR8569]
(ab131254)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-CD27 antibody [EPR8569] (ab131254)

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