abcam

Product datasheet

Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free ab192336

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 11 Images

Overview

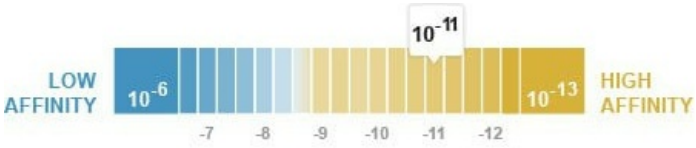
Product name	Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EPR8569] to CD27 - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	ab192336 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 cell-based 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**.

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 7.90 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR8569
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab192336 in the following tested applications.

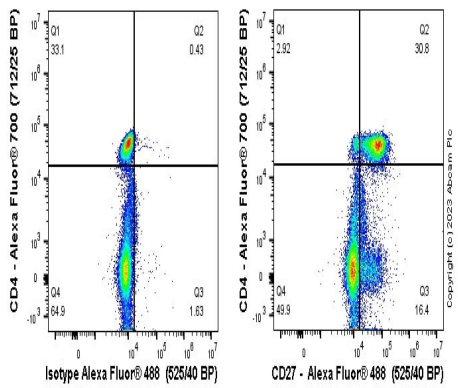
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, 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). Please check the parent abID, **ab131254**, for more information on dilutions.
ICC/IF		Use at an assay dependent concentration.
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

Target

Function	Receptor for CD70/CD27L. May play a role in survival of activated T-cells. May play a role in apoptosis through association with SIVA1.
Tissue specificity	Found in most T-lymphocytes.
Sequence similarities	Contains 3 TNFR-Cys repeats.
Post-translational modifications	Phosphorylated and O-glycosylated.
Cellular localization	Membrane.

Images



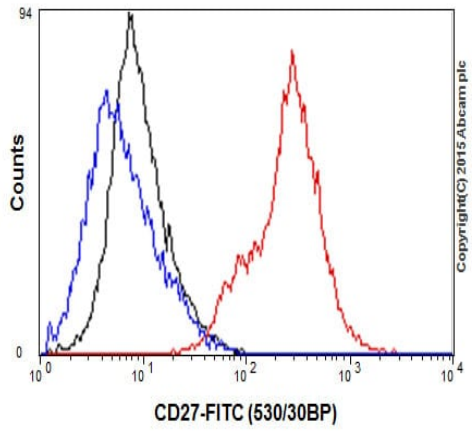
Flow Cytometry (Intracellular) - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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

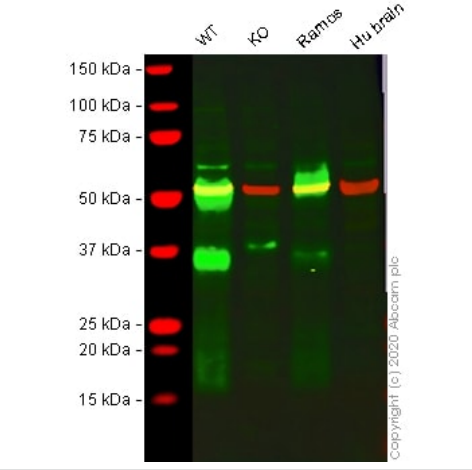
Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.



Flow Cytometry (Intracellular) - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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.

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] - Low endotoxin, Azide free (ab192336)

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.

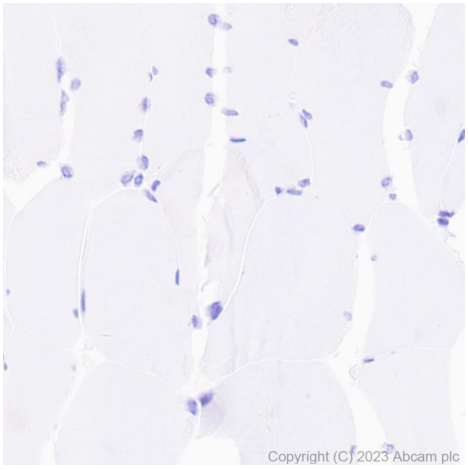
Predicted band size: 29 kDa
Observed band size: 35 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab131254**).

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.



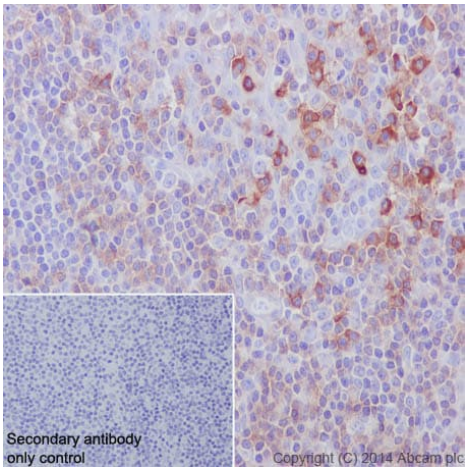
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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 **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.

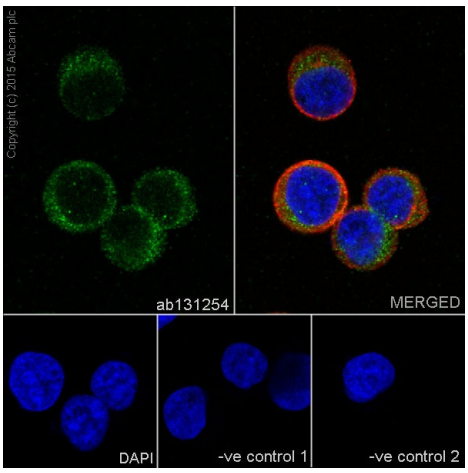
Negative control: No staining on human skeletal muscle. The section was incubated with **ab131254** at 4°C overnight.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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 (**ab131254**).



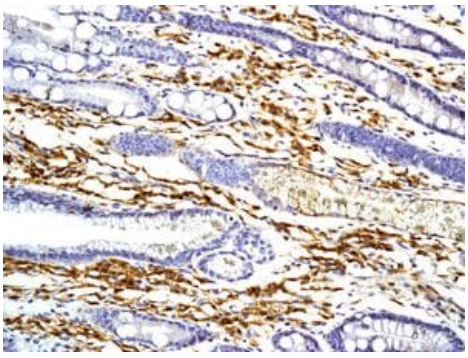
Immunocytochemistry/ Immunofluorescence - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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).

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

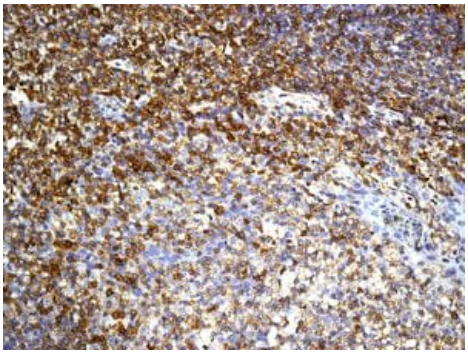


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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 (**ab131254**).

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

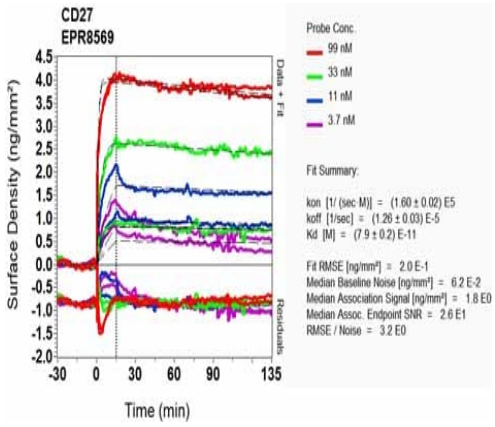


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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**).

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



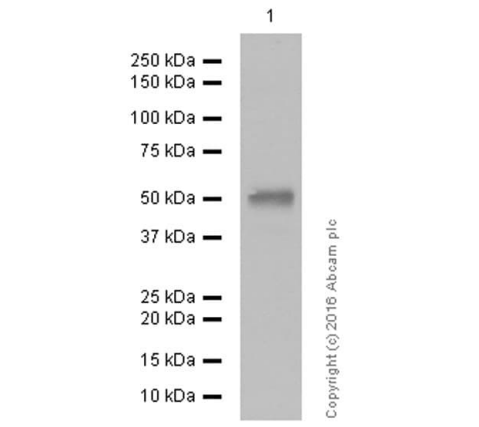
OI-RD Scanning - Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

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] - Low endotoxin, Azide free (ab192336)

Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336) + Human spleen lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

Predicted band size: 29 kDa

Observed band size: 55 kDa

Exposure time: 1 minute

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

Anti-CD27 antibody [EPR8569] - Low endotoxin, Azide free (ab192336)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors