abcam

Product datasheet

Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free ab166865

敲除验证 重组 RabMAb

2 References 15 图像

概述

产品名称	Anti-CD20抗体[EP459Y] - Low endotoxin, Azide free
描述	兔单克隆抗体[EP459Y] to CD20 - Low endotoxin, Azide free
宿主	Rabbit
特异性	For optimal performance in IHC, the primary antibody should be incubated overnight at 4?.
经测试应用	**适用于:** Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P
种属反应性	**与反应:** Human **预测可用于:** Monkey **不与反应:** Mouse, Rat
免疫原	Synthetic peptide within Human CD20 aa 250-350 (C terminal) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary. Database link: **P11836**
阳性对照	WB: Wild-type Raji cell lysate Flow Cyt (intra): Ramos cells
常规说明	ab166865 is the carrier-free version of **ab78237**. 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.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	Constituent: 100% PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	EP459Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab166865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。

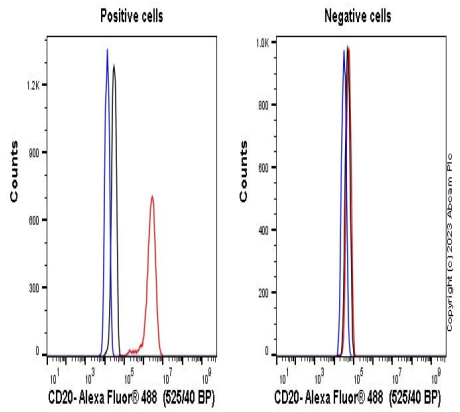
应用	Ab评论	说明
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. For optimal performance in IHC, the primary antibody should be incubated overnight at 4?.

靶标

功能	This protein may be involved in the regulation of B-cell activation and proliferation.
组织特异性	Expressed on B-cells.
疾病相关	Defects in MS4A1 are the cause of immunodeficiency common variable type 5 (CVID5) [MIM:613495]; also called antibody deficiency due to CD20 defect. CVID5 is a primary

	immunodeficiency characterized by antibody deficiency, hypogammaglobulinemia, recurrent bacterial infections and an inability to mount an antibody response to antigen. The defect results from a failure of B-cell differentiation and impaired secretion of immunoglobulins; the numbers of circulating B cells is usually in the normal range, but can be low.
序列相似性	Belongs to the MS4A family.
翻译后修饰	Phosphorylated. Might be functionally regulated by protein kinase(s).
细胞定位	Membrane.

图片



Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).

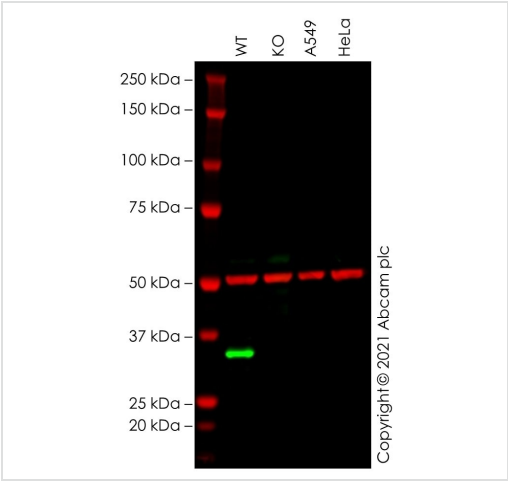
Flow cytometry overlay histogram showing left Ramos positive cells and right negative HEK293 stained with **ab78237** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab78237**) (1x 10^6 in 100µl at 0.2µg/ml (1/2500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Ramos Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Western blot - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

All lanes : Anti-CD20 antibody [EP459Y] - Rat IgG2a (Chimeric) (**ab279300**) at 1/1000 dilution

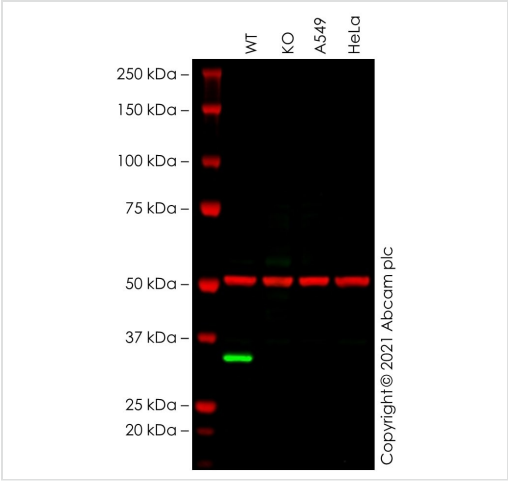
Lane 1 : Wild-type Raji cell lysate
Lane 2 : MS4A1 knockout Raji cell lysate
Lane 3 : A549 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 33 kDa

False colour image of Western blot: Anti-CD20 antibody [EP459Y] - Rat IgG2a staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279300** was shown to bind specifically to CD20. A band was observed at 33 kDa in wild-type Raji cell lysates with no signal observed at this size in MS4A1 knockout cell line **ab273871** (knockout cell lysate **ab263259**). To generate this image, wild-type and MS4A1 knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rat IgG H&L (IRDye® 800CW) preabsorbed (**ab253031**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

All lanes : Anti-CD20 antibody [EP459Y] - Mouse IgG2a (Chimeric) (**ab279299**) at 1/1000 dilution

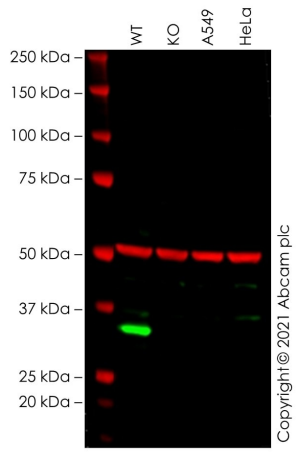
Lane 1 : Wild-type Raji cell lysate
Lane 2 : MS4A1 knockout Raji cell lysate
Lane 3 : A549 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 33 kDa

False colour image of Western blot: Anti-CD20 antibody [EP459Y] - Mouse IgG2a staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279299** was shown to bind specifically to CD20. A band was observed at 33 kDa in wild-type Raji cell lysates with no signal observed at this size in MS4A1 knockout cell line **ab273871** (knockout cell lysate **ab263259**). To generate this image, wild-type and MS4A1 knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

All lanes : Anti-CD20 antibody [EP459Y] - Mouse IgG1 (Chimeric) (**ab279298**) at 1/1000 dilution

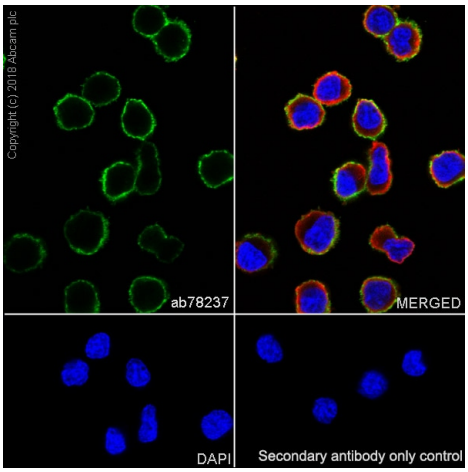
Lane 1 : Wild-type Raji cell lysate
Lane 2 : MS4A1 knockout Raji cell lysate
Lane 3 : A549 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 33 kDa

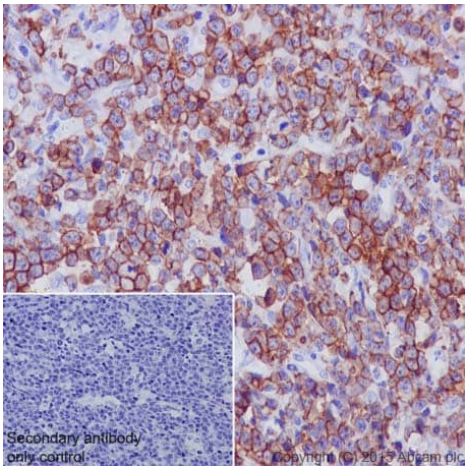
False colour image of Western blot: Anti-CD20 antibody [EP459Y] - Mouse IgG1 staining at 1/1000 dilution, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab279298** was shown to bind specifically to CD20. A band was observed at 33 kDa in wild-type Raji cell lysates with no signal observed at this size in MS4A1 knockout cell line **ab273871** (knockout cell lysate **ab263259**). To generate this image, wild-type and MS4A1 knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween$^{Â®}$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 Â°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye$^{Â®}$ 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye$^{Â®}$ 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Immunocytochemistry/ Immunofluorescence analysis of Ramos (human Burkitt's lymphoma B lymphocyte) labeling CD20 with purified **ab78237** at 1/10 dilution. Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/1000 was used as the secondary antibody. Cells were counterstained with **ab195889**, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200. PBS instead of the primary antibody was used as negative control. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Nuclei were stained with DAPI (blue).

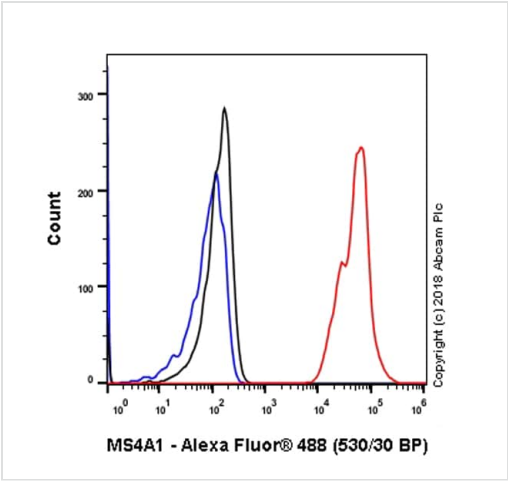
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

This IHC data was generated using the same anti-CD20 antibody clone, EP459Y, in a different buffer formulation (cat# **ab78237**).

Immunohistochemical staining of paraffin embedded human B cell lymphoma with purified **ab78237** at a working dilution of 1/50. The secondary antibody used is **ab97051**, a goat anti-rabbit IgG (H&L) at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed 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.



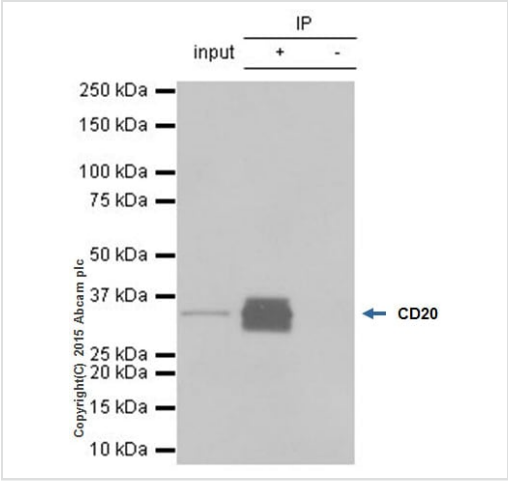
Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Intracellular Flow Cytometry analysis ofRamos (Human Burkitt's lymphoma B lymphocyte) labeling CD20 with purified **ab78237** at 1/200 dilution (Red). Goat anti rabbit IgG (Alexa Fluor®488, **ab150081**) at 1/2000 dilution was used as secondary antibody. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol.

Isotype control: Rabbit monoclonal IgG (**ab172730**) (Black)

Unlabelled cells: (Blue)

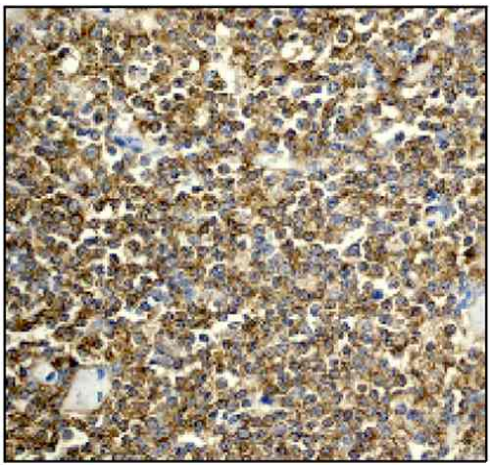
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunoprecipitation - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

ab78237 (purified) at 1/20 immunoprecipitating CD20 in 10 µg Ramos cell lysate (Lanes 1 and 2, observed at 33 kDa). Lane 3 - Rabbit monoclonal IgG (**ab172730**). For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution. Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST

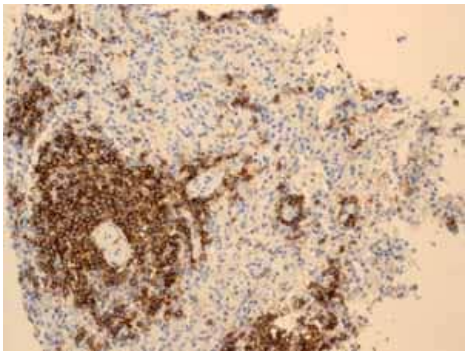
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Unpurified **ab78237** staining human CD20 in human lymphoma tissue by immunohistochemistry using formalin fixed, paraffin embedded tissue.

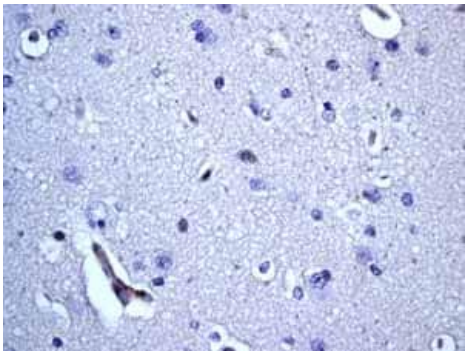
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Unpurified **ab78237** showing positive staining in normal human spleen tissue.

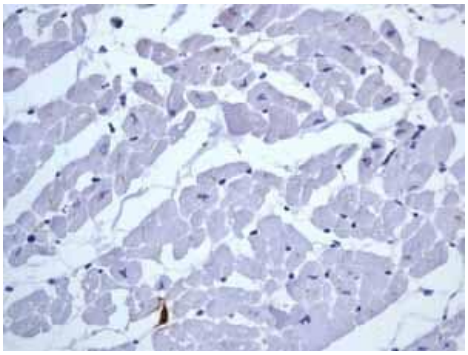
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Unpurified **ab78237** showing negative staining in normal human brain tissue.

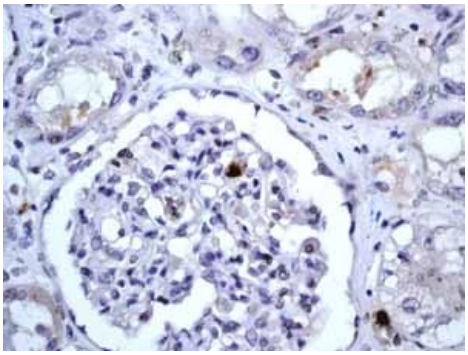
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Unpurified **ab78237** showing negative staining in normal human heart tissue.

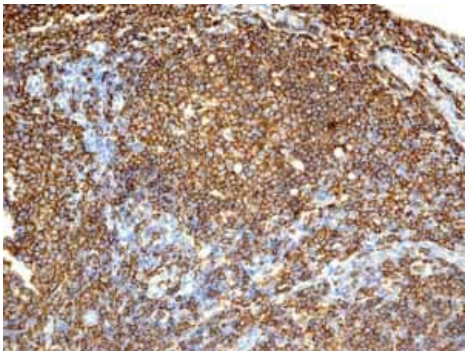
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

Unpurified **ab78237** showing negative staining in normal human kidney tissue.

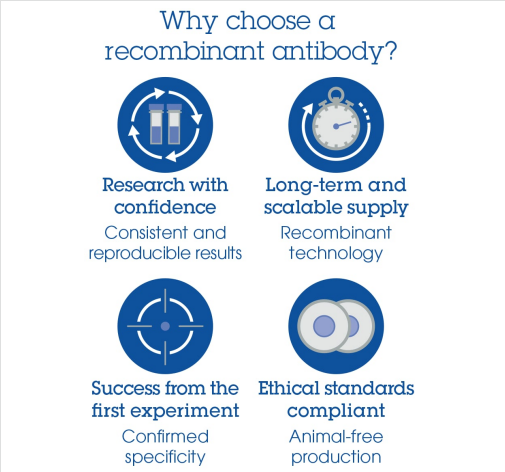
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab78237**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

This IHC data was generated using the same anti-CD20 antibody clone, EP459Y, in a different buffer formulation (cat# **ab78237**).

Unpurified **ab78237** showing positive staining in normal human tonsil tissue.



Anti-CD20 antibody [EP459Y] - Low endotoxin, Azide free (ab166865)

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