


Product datasheet

Anti-CD20 antibody [EP459Y] ab78237

KO VALIDATED Recombinant RabMAb

★★★★★ 7 Abreviews 59 References 18 Images

Overview

Product name	Anti-CD20 antibody [EP459Y]
Description	Rabbit monoclonal [EP459Y] to CD20
Host species	Rabbit
Specificity	For optimal performance in IHC, the primary antibody should be incubated overnight at 4°.
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P
Species reactivity	Reacts with: Human Predicted to work with: Monkey  Does not react with: Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human B cell lymphoma tissue. Human spleen and tonsil tissue. IP: Ramos whole cell lysate. WB: Raji whole cell lysate; Wild-type Raji cell lysate ICC/IF: Ramos cells Flow Cyt (intra): Ramos cells
General notes	<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>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP459Y
Isotype	IgG

Applications

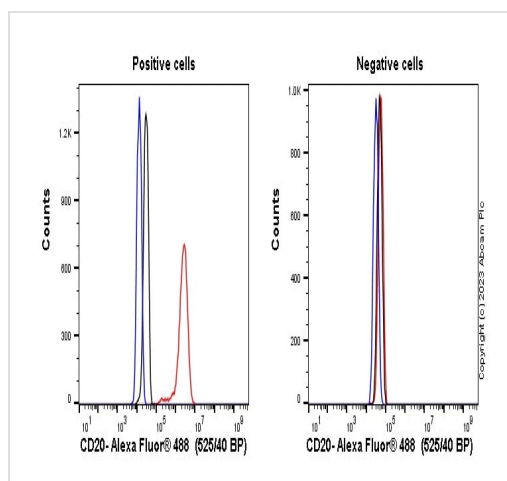
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab78237 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/200. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (1)	1/10.
WB		1/1000 - 1/10000. Detects a band of approximately 33 kDa (predicted molecular weight: 33 kDa).
IP		1/20.
IHC-P	★★★★★ (5)	1/50 - 1/250. 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°.

Target

Function	This protein may be involved in the regulation of B-cell activation and proliferation.
Tissue specificity	Expressed on B-cells.
Involvement in disease	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.
Sequence similarities	Belongs to the MS4A family.
Post-translational modifications	Phosphorylated. Might be functionally regulated by protein kinase(s).
Cellular localization	Membrane.

Images



Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] (ab78237)

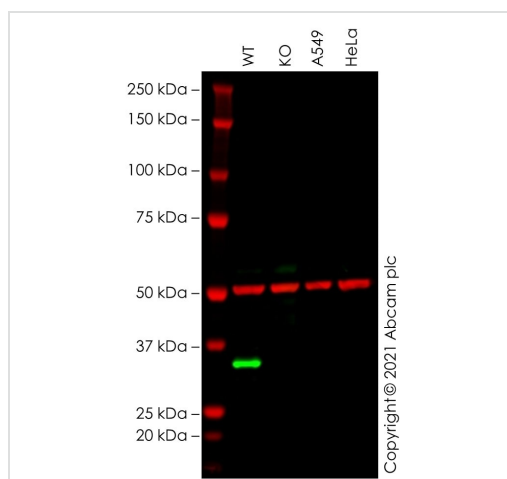
Flow cytometry overlay histogram showing left Ramos positive cells and right negative HEK293 stained with ab78237 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10µg/ml human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

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.

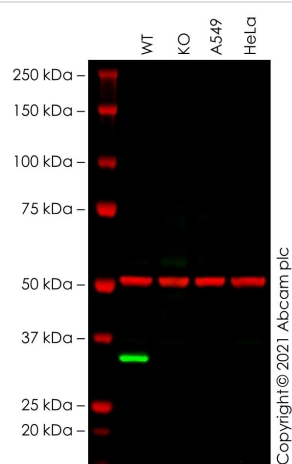
Performed under reducing conditions.

Predicted band size: 33 kDa

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]
(ab78237)

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.

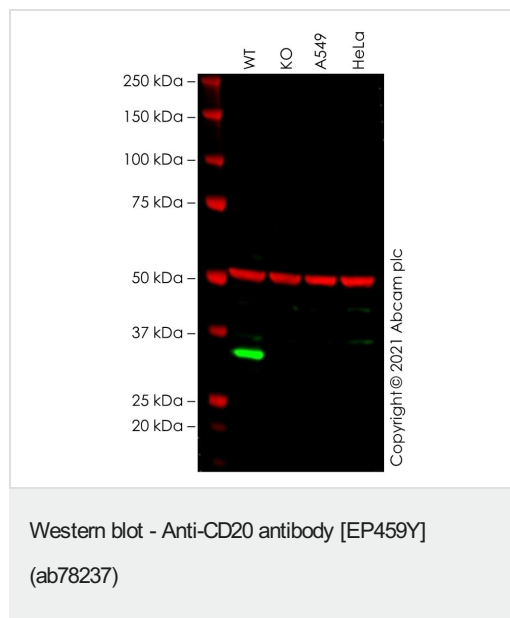
Performed under reducing conditions.

Predicted band size: 33 kDa

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.



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

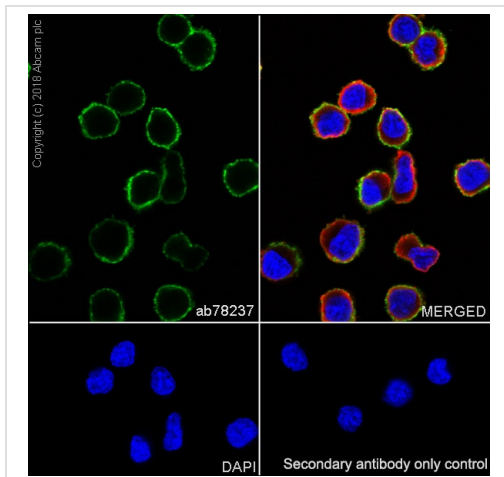
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 33 kDa

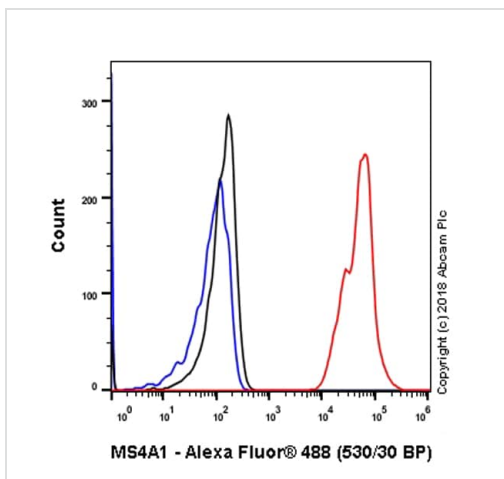
Observed band size: 33 kDa

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® 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.



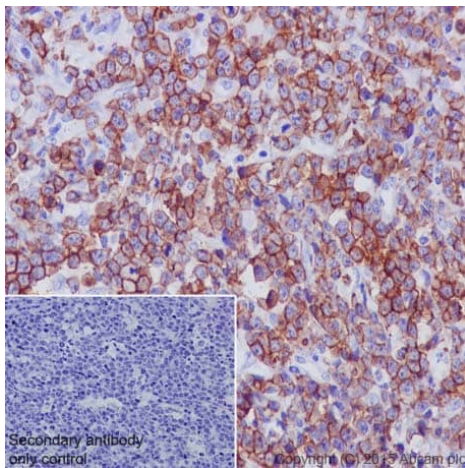
Immunocytochemistry/ Immunofluorescence - Anti-CD20 antibody [EP459Y] (ab78237)

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)



Flow Cytometry (Intracellular) - Anti-CD20 antibody [EP459Y] (ab78237)

Intracellular Flow Cytometry analysis of Ramos (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)

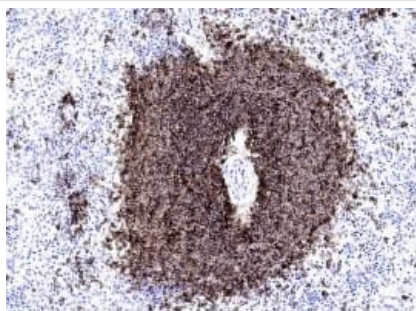


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)

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 counterstained with hematoxylin. Antigen retrieval was performed 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.

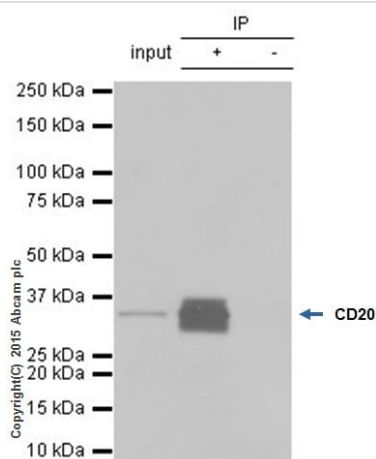


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)

Image courtesy of an abreview by Mr Carl Hobbs

Formaldehyde-fixed, paraffin-embedded human spleen tissue stained for CD20 using ab78237 at 1/50 dilution in immunohistochemical analysis.

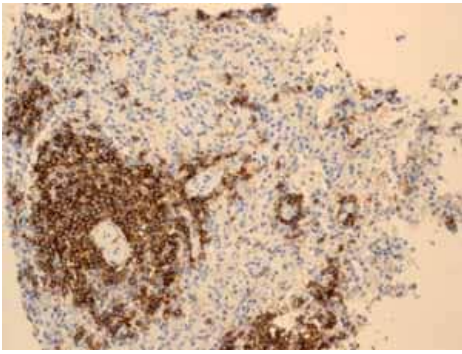
Heat mediated antigen retrieval with citrate pH 6.0.



Immunoprecipitation - Anti-CD20 antibody [EP459Y] (ab78237)

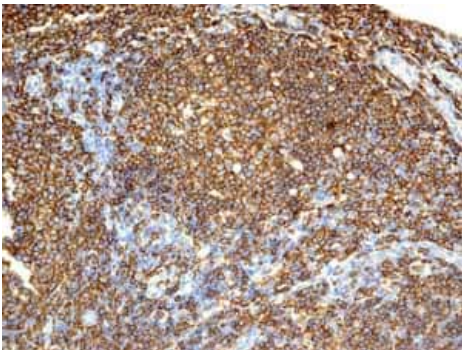
ab78237 (purified) at 1/20 immunoprecipitating CD20 in 10 µg Ramos (Human Burkitt's lymphoma cell line) cell lysate (**Lanes 1 and 2**, observed at 33 kDa). **Lane 3** - Rabbit monoclonal IgG ([ab172730](#)). For western blotting, VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/10,000 dilution.

Blocking/Dilution buffer and concentration: 5% NFDM/TBST.



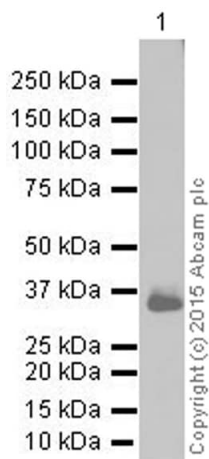
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)

Unpurified ab78237 showing positive staining in human spleen tissue.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)

Unpurified ab78237 showing positive staining in human tonsil tissue.



Western blot - Anti-CD20 antibody [EP459Y] (ab78237)

Anti-CD20 antibody [EP459Y] (ab78237) at 1/10000 dilution (purified) + Raji (Human Burkitt's lymphoma cell line) whole cell lysate

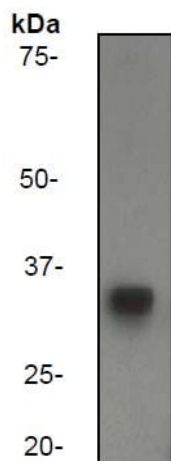
Secondary

Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 33 kDa

Observed band size: 33 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

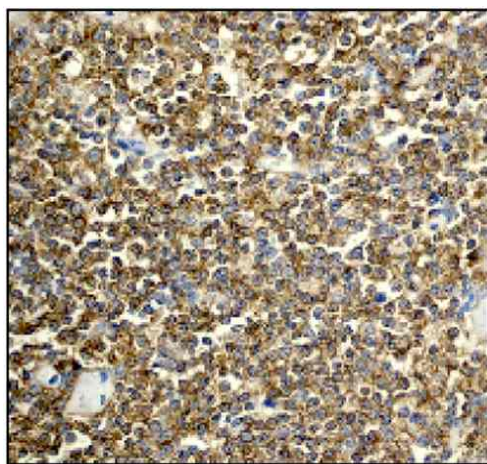


Western blot - Anti-CD20 antibody [EP459Y]
(ab78237)

Anti-CD20 antibody [EP459Y] (ab78237) at 1/5000 dilution
(unpurified) + Raji (Human Burkitt's lymphoma cell line) cell lysate

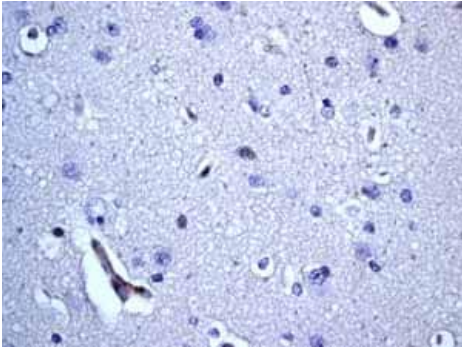
Predicted band size: 33 kDa

Observed band size: 33 kDa



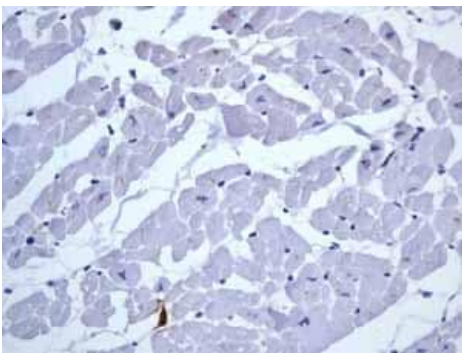
Immunohistochemistry (Formalin/PFA-fixed paraffin-
embedded sections) - Anti-CD20 antibody [EP459Y]
(ab78237)

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



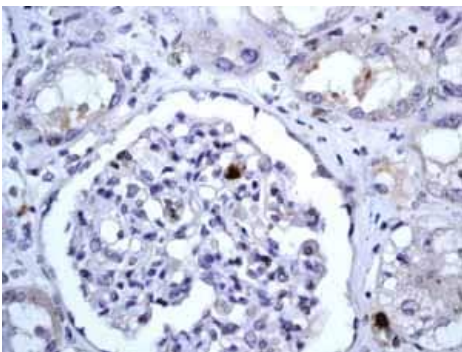
Unpurified ab78237 showing **negative staining** in human brain tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)



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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)



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

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD20 antibody [EP459Y] (ab78237)

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-CD20 antibody [EP459Y] (ab78237)

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