abcam

ab237772

Anti-CD19 antibody [SP291] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	SP291
Purification technique	Affinity purification Protein A/G
Concentration	1.038 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS by protein A/G.

Reactivity data

mIHC

Tested

Species	Human
Dilution info	1/5000

Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Expected

Species	Serinus canarius, Cat, Chicken, Horse, Llama, Pigeon
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Serinus canarius, Cat, Chicken, Horse, Llama, Pigeon
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat mediated antigen retrieval with EDTA buffer pH 8.0 before commencing with IHC staining protocol.

Species	Serinus canarius
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Cat

Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Chicken
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Horse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Llama
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Pigeon
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Serinus canarius, Cat, Chicken, Horse, Llama, Pigeon
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information CD19

Function	Functions as a coreceptor for the B-cell antigen receptor complex (BCR) on B-lymphocytes (PubMed:29523808). Decreases the threshold for activation of downstream signaling pathways and for triggering B-cell responses to antigens (PubMed:1373518, PubMed:16672701, PubMed:2463100). Activates signaling pathways that lead to the activation of phosphatidylinositol 3-kinase and the mobilization of intracellular Ca(2+) stores (PubMed:12387743, PubMed:16672701, PubMed:9317126, PubMed:9382888). Is not required for early steps during B cell differentiation in the blood marrow (PubMed:9317126). Required for normal differentiation of B-1 cells (By similarity). Required for normal B cell differentiation and proliferation in response to antigen challenges (PubMed:1373518, PubMed:2463100). Required for normal levels of serum immunoglobulins, and for production of high-affinity antibodies in response to antigen challenge (PubMed:12387743, PubMed:16672701, PubMed:9317126).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab237772 is the carrier-free version of ab227688.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD19 is a transmembrane protein also known as B4 or B-lymphocyte antigen CD19 with an approximate molecular weight of 95 kDa. It is expressed on the surface of B cells throughout their development from pro-B cells to mature B cells. CD19 functions mechanically as a coreceptor enhancing the sensitivity of B cell receptor (BCR) signaling. This protein plays a significant role in promoting the activation and proliferation of B cells by lowering the threshold for antigen receptor engagement.
Biological function summary	CD19 acts as an essential player in B cell signaling. CD19 integrates signals from the BCR with other coreceptors acting as a signaling hub. It participates in forming a signaling complex that includes proteins like CD21 and CD81 which further amplifies BCR-mediated signaling. This complex plays a critical role in B cell activation differentiation and survival ensuring that immune responses are efficiently mounted against pathogens.
Pathways	The CD19 protein fits into key immune response pathways such as the BCR signaling pathway and the PI3K-Akt signaling pathway. CD19 collaborates with other proteins like CD21 CD81 and PI3K within these pathways. Its interaction in the PI3K-Akt pathway for instance supports critical processes including B cell activation and homeostasis contributing to the adaptive immune response.
Associated diseases and disorders	CD19 is connected to B cell malignancies and autoimmune diseases. B cell acute lymphoblastic leukemia (ALL) often shows aberrant CD19 expression making it a valuable target for therapeutic interventions like anti-CD19 monoclonal antibodies. Additionally overexpression or mutations of CD19 may contribute to autoimmune diseases by disrupting normal B cell regulation. CD19's connection with proteins like CD22 and CD20 in these pathological contexts highlights its relevance in developing targeted therapies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

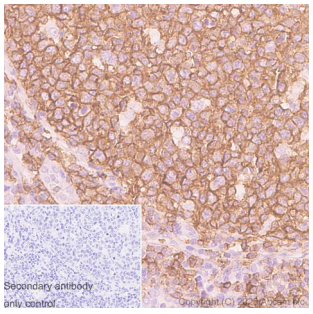
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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30 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

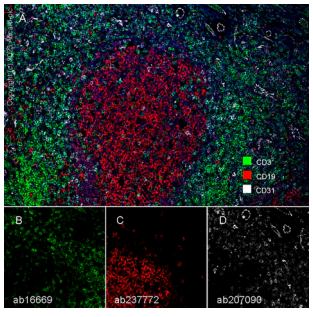
This data was developed using ab227688, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD19 with ab227688 at 1/100 (0.15 ug/ml) dilution, followed by a ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Positive staining in human tonsil.
The section was incubated with ab227688 for 10 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.

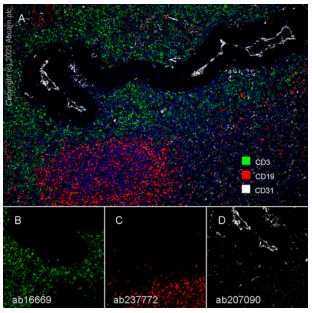


Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Multiplex immunohistochemistry staining using rabbit Anti-CD19 antibody

This image was generated using ab227688, the same clone, but with a different buffer formulation. Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI. Panel B: anti-CD3 epsilon stained on T cells with ab16669 at 1/500 dilution Panel C: anti-CD19 stained on B cells with ab237772 at 1/5000 dilution Panel D: anti-CD31 stained on endothelial cells and immune cell

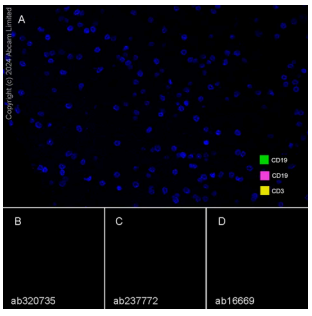
subsets with ab207090 at 1/500 dilution The section was incubated in three rounds of staining: in the order of ab207090 and ab16669 for 30 mins, then ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

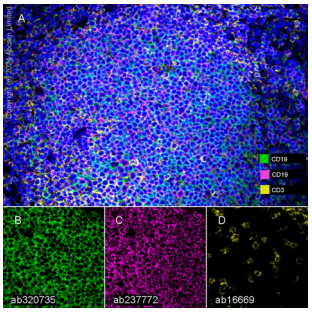
CD19 Multiplex immunohistochemistry staining using rabbit Anti-CD19 antibody

Panel A: merged staining of anti-CD31 (gray; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, nuclear counterstain was DAPI. Panel B: anti-CD3 epsilon stained on T cells with ab16669 at 1/500 dilution Panel C: anti-CD19 stained on B cells with ab237772 at 1/5000 dilution Panel D: anti-CD31 stained on endothelial cells with ab207090 at 1/500 dilution The section was incubated in three rounds of staining: in the order of ab207090 and ab16669 for 30 mins, then ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins"



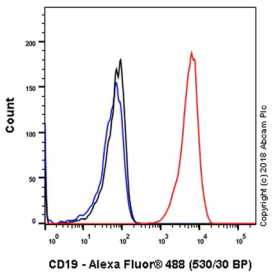
Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Panel A: merged staining of anti-CD19 (green; Opal™520), anti-CD19 (green; Opal™570) and anti-CD3 (yellow; Opal™690) on human cerebrum. Panel B: anti-CD19 showed no staining in human cerebrum. Panel C: anti-CD19 showed no staining in human cerebrum. Panel D: anti-CD3 showed no staining in human cerebrum. Nuclear DNA was labeled with DAPI (shown in blue). The section was incubated in three rounds of staining: in the order of ab320735 at 1/2000 dilution, ab237772 at 1/5000 dilution and ab16669 at 1/150 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary used was Opal Polymer HRP Ms + Rb. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

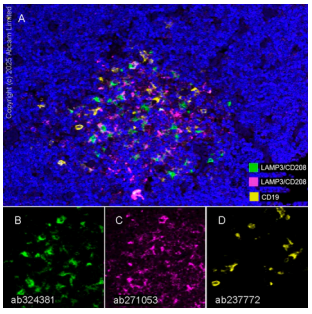
Panel A: merged staining of anti-CD19 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on human tonsil. Panel B: anti-CD19 staining the B lymphocytes in human tonsil. Panel C: anti-CD19 staining the B lymphocytes in human tonsil. Panel D: anti-CD3 staining the T lymphocytes in human tonsil. Nuclear DNA was labeled with DAPI (shown in blue). The section was incubated in three rounds of staining: in the order of ab320735 at 1/2000 dilution, ab237772 at 1/5000 dilution and ab16669 at 1/150 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary used was Opal Polymer HRP Ms + Rb. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Flow Cytometry (Intracellular) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Flow Cytometry (Intracellular) staining using rabbit Anti-CD19 antibody

Intracellular Flow Cytometry analysis of Raji (human Burkitt's lymphoma) labeling CD19 with purified ab227688 at 1/20 dilution (7.5µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as a secondary antibody. Isotypecontrol - Rabbit monoclonal IgG (ab172730) (black). Unlableled control - Unlabelled cells (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227688).



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Multiplex immunohistochemistry staining of Human thymus using rabbit Anti-CD19 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human thymus tissue staining CD208 with ab324381 at a 1:100 (4.94 ug/ml) dilution, ab281573 anti-LAMP3/CD208 used at 1:500 (0.938 ug/ml) dilution and ab237772 anti-CD19 used at a 1:5000 (0.21 ug/ml) dilution.

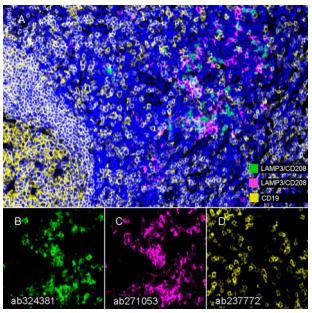
Panel A: merged staining of anti-LAMP3/CD208 (green; Opal™520), anti-LAMP3/CD208 (magenta; Opal™570) and anti-CD19 (yellow; Opal™690) on human thymus.
Panel B: anti-LAMP3/CD208 staining dendritic cells in human thymus.
Panel C: anti-LAMP3/CD208 staining dendritic cells in human thymus.
Panel D: anti-CD19 staining B lymphocytes of human thymus.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab324381, ab271053, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Multiplex immunohistochemistry staining of Human tonsil using rabbit Anti-CD19 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human tonsil tissue staining CD208 with ab324381 at a 1:100 (4.94 ug/ml) dilution, ab281573 anti-LAMP3/CD208 used at 1:500 (0.938 ug/ml) dilution and ab237772 anti-CD19 used at a 1:5000 (0.21 ug/ml) dilution.

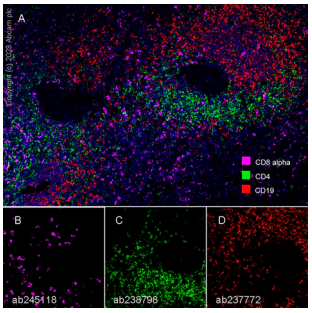
Panel A: merged staining of anti-LAMP3/CD208 (green; Opal™520), anti-LAMP3/CD208 (magenta; Opal™570) and anti-CD19 (yellow; Opal™690) on human tonsil.
Panel B: anti-LAMP3/CD208 staining dendritic cells in human tonsil.
Panel C: anti-LAMP3/CD208 staining dendritic cells in human tonsil.
Panel D: anti-CD19 staining B lymphocytes of human tonsil.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab324381, ab271053, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

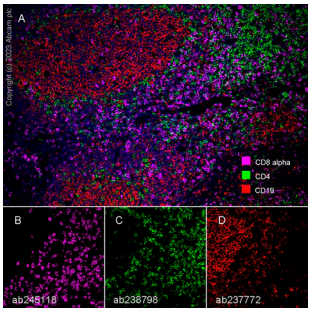
CD19 Multiplex immunohistochemistry staining of Human spleen using rabbit Anti-CD19 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human spleen tissue labeling CD8 alpha with ab245118 at 1/500 dilution, CD4 with ab238798 at 1/500, and CD19 with ab237772 at 1/5000 dilution.
Panel A: merged staining of anti-CD8 alpha (magenta; Opal™690), anti-CD4 (green; Opal™520) and anti-CD19 (red; Opal™570) on human spleen.
Panel B: anti-CD8 alpha stained on cytotoxic T cells.
Panel C: anti-CD4 stained on T helper cells.
Panel D: anti-CD19 stained on B cells.

Sections were treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins before antibody incubation. The section was incubated in three rounds of staining: in the order of ab245118 for 30 mins, then ab238798 and ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

DAPI was used as a nuclear counterstain.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

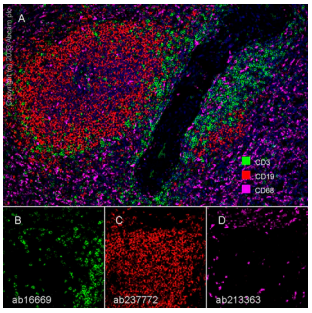
CD19 Multiplex immunohistochemistry staining of Human tonsil using rabbit Anti-CD19 antibody

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded Human tonsil tissue labeling CD8 alpha with ab245118 at 1/500 dilution, CD4 with ab238798 at 1/500, and CD19 with ab237772 at 1/5000 dilution.
Panel A: merged staining of anti-CD8 alpha (magenta; Opal™690), anti-CD4 (green; Opal™520) and anti-CD19 (red; Opal™570) on human tonsil.
Panel B: anti-CD8 alpha stained on cytotoxic T cells.
Panel C: anti-CD4 stained on T helper cells.
Panel D: anti-CD19 stained on B cells.

Sections were treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins before antibody incubation. The section was incubated in three rounds of staining: in the order of ab245118 for 30 mins, then ab238798 and ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

DAPI was used as a nuclear counterstain.

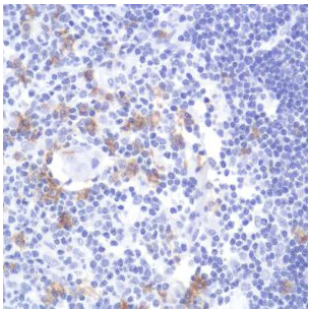
The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Multiplex immunohistochemistry staining using rabbit Anti-CD19 antibody

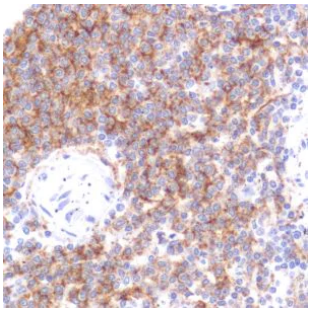
This image was generated using ab227688, the same clone, but with a different buffer formulation. Panel A: merged staining of anti-CD68 (magenta; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human spleen. Secondary antibody was Opal Polymer HRP Ms + Rb, and couterstaining was with DAPI. Panel B: anti-CD3 epsilon stained on T cells with ab16669 at 1/500 dilution Panel C: anti-CD19 stained on B cells with ab237772 at 1/5000 dilution Panel D: anti-CD68 stained on macrophages with ab213363 1/500 dilution The section was incubated in three rounds of staining: in the order of ab213363 and ab16669 for 30 mins, then ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD19 antibody

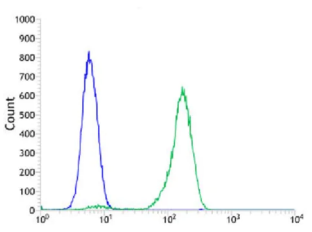
Formalin-fixed, paraffin-embedded human thymus tissue stained for CD19 using ab227688 at 1/100 dilution in immunohistochemical analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227688).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD19 antibody

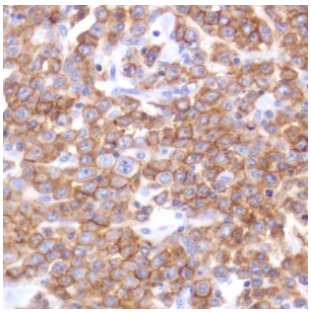
Formalin-fixed, paraffin-embedded human spleen tissue stained for CD19 using ab227688 at 1/100 dilution in immunohistochemical analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227688).



Flow Cytometry (Intracellular) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Flow Cytometry (Intracellular) staining using rabbit Anti-CD19 antibody

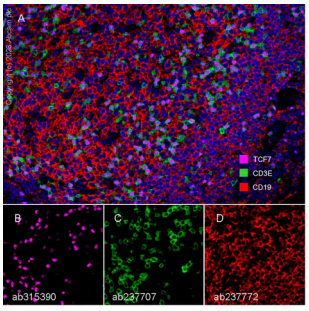
Intracellular Flow Cytometry analysis of Ramos (human Burkitt's lymphoma cell line) cells, labeling CD19 with ab227688 at 1/400 dilution (green) compared to a Rabbit IgG negative control (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227688).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD19 antibody

Formalin-fixed, paraffin-embedded human B-Cell lymphoma tissue stained for CD19 using ab227688 at 1/100 dilution in immunohistochemical analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227688).



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human tonsil tissue staining TCF7 with ab315390 at a 1:2000 (0.26 ug/ml) dilution, ab237707 anti-CD3E used at 1:500 (1.01 ug/ml) dilution and ab237772 anti-CD19 used at a 1:5000 (0.21 ug/ml) dilution.

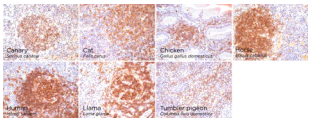
Panel A: merged staining of anti-TCF7 (magenta; Opal™690), anti-CD3E (green; Opal™520) and anti-CD19 (red; Opal™570) on human tonsil.
Panel B: anti-TCF7 staining T lymphocytes of human tonsil.
Panel C: anti-CD3E staining T lymphocytes of human tonsil.
Panel D: anti-CD19 staining B lymphocytes of human tonsil.

The section was incubated in three rounds of staining: in the order of ab315390, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

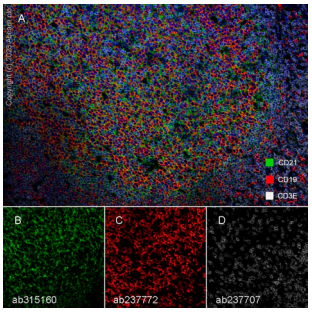


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-CD19 antibody

This data was developed using ab227688, the same antibody clone in a different buffer formulation
The BOND™ Polymer Refine Detection System on the BOND RX Automated Stainer (DS9800, Leica Biosystems, Wetzlar, Germany) was used for immunohistochemical labelling of formalin-fixed, paraffin-embedded (FFPE) tissues. The protocol includes a peroxide block, post-primary reagent, polymer detection, DAB chromogen, and haematoxylin counterstain, all automated to minimize variability. Antigen retrieval was performed by heat-induced epitope retrieval (HIER) for 20

minutes in Tris-EDTA buffer (BOND Epitope Retrieval Solution 2, AR9640, Leica Biosystems, Wetzlar, Germany). For mouse and rabbit antibodies, the sequence comprised peroxide block (10 min), primary antibody incubation (30 min), post-primary (10 min), polymer (10 min), DAB (10 min), and haematoxylin (8 min). For rat antibodies, an anti-rat step (1:300 dilution, Vector Laboratories, California, USA; 20 min) was included prior to polymer application (15 min).



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human tonsil tissue staining CD21 with ab315160 at a 1:50 (10.56 ug/ml) dilution, ab237772 anti-CD19 used at 1:5000 (0.21 ug/ml) dilution and ab237707 anti-CD3E used at a 1:500 (1.01 ug/ml) dilution.

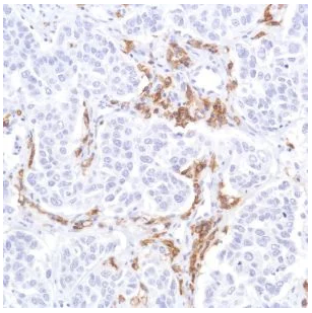
Panel A: merged staining of anti-CD21 (green; Opal™690), anti-CD3E (grey; Opal™520) and anti-CD19 (red; Opal™570) on human tonsil.
Panel B: anti-CD21 staining mature B lymphocytes and T-lymphocytes of human tonsil.
Panel C: anti-CD19 staining B lymphocytes of human tonsil.
Panel D: anti-CD3E staining T lymphocytes of human tonsil.

The section was incubated in three rounds of staining: in the order of ab315160, ab237772, and ab237707 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

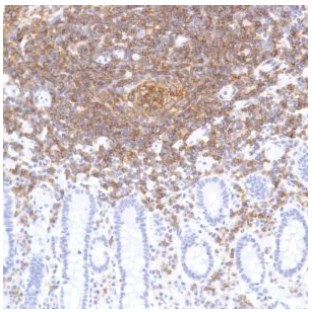
Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



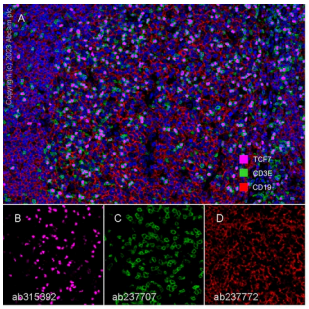
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Formalin-fixed, paraffin-embedded human breast ductal carcinoma tissue stained for CD19 using ab227688 at 1/100 dilution in immunohistochemical analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, and sodium azide (ab227688).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Formalin-fixed, paraffin-embedded human colon tissue stained for CD19 using ab227688 at 1/100 dilution in immunohistochemical analysis.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227688).



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human tonsil tissue staining TCF7 with ab315392 at a 1:2000 (0.26 ug/ml) dilution, ab237707 anti-CD3E used at 1:500 (1.01 ug/ml) dilution and ab237772 anti-CD19 used at a 1:5000 (0.21 ug/ml) dilution.

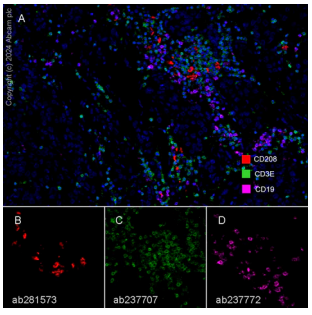
Panel A: merged staining of anti-TCF7 (magenta; Opal™690), anti-CD3E (green; Opal™520) and anti-CD19 (red; Opal™570) on human tonsil.
Panel B: anti-TCF7 staining T lymphocytes of human tonsil.
Panel C: anti-CD3E staining T lymphocytes of human tonsil.
Panel D: anti-CD19 staining B lymphocytes of human tonsil.

The section was incubated in three rounds of staining: in the order of ab315392, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Nuclear counter stain with DAPI.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human liver cancer labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human liver cancer.

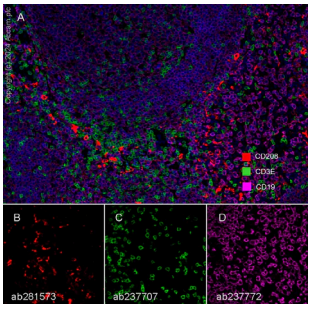
Panel B: anti-CD208 staining dendritic cells in human liver cancer.

Panel C: anti-CD3E staining T lymphocytes in human liver cancer.

Panel D: anti-CD19 staining B lymphocytes of human liver cancer.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

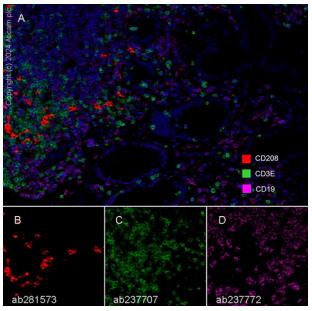
CD19 Multiplex immunohistochemistry staining of Human tonsil tissue using rabbit Anti-CD19 antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human tonsil.
Panel B: anti-CD208 staining dendritic cells in human tonsil.
Panel C: anti-CD3E staining T lymphocytes in human tonsil.
Panel D: anti-CD19 staining B lymphocytes of human tonsil.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human colon.

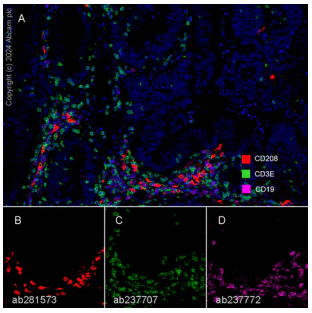
Panel B: anti-CD208 staining dendritic cells in human colon.

Panel C: anti-CD3E staining T lymphocytes in human colon.

Panel D: anti-CD19 staining B lymphocytes of human colon.

The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon cancer labelling CD208 with ab281573 at 1/1200 (B), CD3E with ab237707 at 1/1200 dilution (C) and CD19 with ab237772 at 1/1200 dilution(D). Opal Polymer HRP Ms + Rb was used as a secondary antibody, and DAPI was used for a nuclear counter stain. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Panel A: merged staining of anti-CD208 (red; Opal™570), anti-CD3E (green; Opal™520) and anti-CD19 (magenta; Opal™690) on human colon cancer.

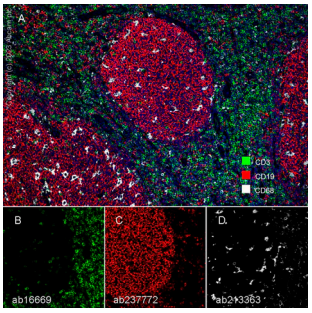
Panel B: anti-CD208 staining dendritic cells in human colon cancer.

Panel C: anti-CD3E staining T lymphocytes in human colon cancer.

Panel D: anti-CD19 staining B lymphocytes of human colon cancer.

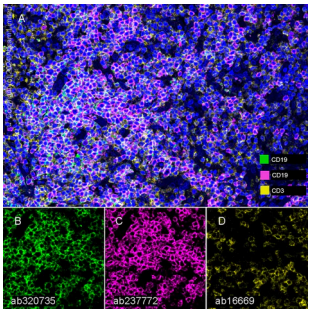
The section was incubated in three rounds of staining: in the order ab281573, ab237707, and ab237772 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

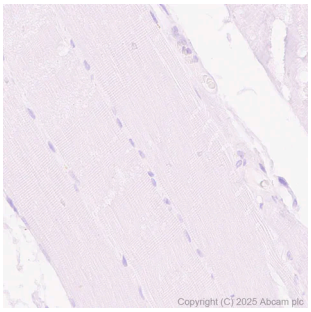
This data was developed using ab16669, the same antibody clone in a different buffer formulation. Panel A: merged staining of anti-CD68 (gray; Opal™690), anti-CD3 epsilon (green; Opal™520) and anti-CD19 (red; Opal™570) on Formalin/PFA-fixed paraffin-embedded sections of human tonsil. Secondary antibody was Opal Polymer HRP Ms + Rb, and counterstaining was with DAPI. Panel B: anti-CD3 epsilon stained on T cells with ab16669 at 1/500 dilution Panel C: anti-CD19 stained on B cells with ab237772 at 1/5000 dilution Panel D: anti-CD68 stained on macrophages with ab213363 1/500 dilution The section was incubated in three rounds of staining: in the order of ab213363 and ab16669 for 30 mins, then ab237772 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

Panel A: merged staining of anti-CD19 (green; Opal™520), anti-CD19 (magenta; Opal™570) and anti-CD3 (yellow; Opal™690) on human Hodgkin's lymphoma. Panel B: anti-CD19 staining the B lymphocytes in human Hodgkin's lymphoma. Panel C: anti-CD19 staining the B lymphocytes in human Hodgkin's lymphoma. Panel D: anti-CD3 staining the T lymphocytes in human Hodgkin's lymphoma. Nuclear DNA was labeled with DAPI (shown in blue). The section was incubated in three rounds of staining: in the order of ab320735 at 1/2000 dilution, ab237772 at 1/5000 dilution and ab16669 at 1/150 dilution for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The secondary used was Opal Polymer HRP Ms + Rb. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. The immunostaining was performed on a Leica Biosystems BOND® RX

instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

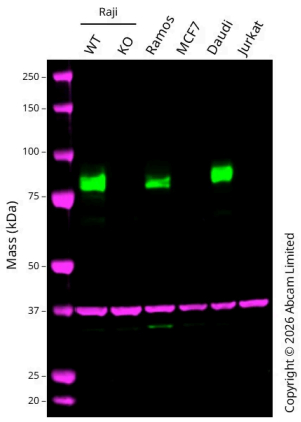
This data was developed using ab227688, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human skeletal muscle labeling CD19 with ab227688 at 1/100 (0.15 ug/ml) dilution, followed by a ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Negative control: no staining in human skeletal muscle.
The section was incubated with ab227688 for 10 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins



Western blot - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Western blot staining using rabbit Anti-CD19 antibody

This data was developed using ab227688, the same antibody clone in a different buffer formulation.

Western blot: Anti-CD19 antibody [SP291] - C-terminal ab227688 staining at 1/400 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 80-90 kDa in Wild-type Raji cell lysates with no signal observed at this size in CD19 knockout Raji cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% 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-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-CD19 antibody [SP291] - C-terminal (ab227688) at 1/400 dilution

Lane 1:
Wild-type Raji at 20 µg

Lane 2:
Western blot - Human CD19 knockout Raji cell line (ab274913) at 20 µg

Lane 3:
Ramos at 20 µg

Lane 4:
MCF7 at 20 µg

Lane 5:
Daudi at 20 µg

Lane 6:
Jurkat at 20 µg

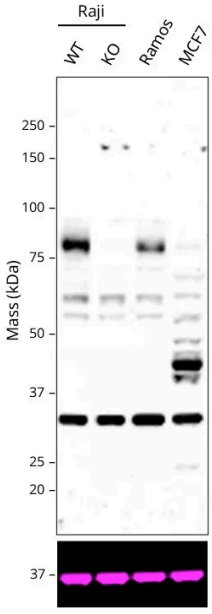
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 80-90 kDa

Observed band size: 80-90 kDa



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Western blot - Anti-CD19 antibody [SP291] - BSA and Azide free (ab237772)

CD19 Western blot staining using rabbit Anti-CD19 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab305999).

Western blot: HRP Anti-CD19 antibody [SP291] (C-terminal) ab305999 staining at 1/1000 dilution, shown in black; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 80 kDa in Wild-type Raji cell line with no signal observed at this size in CD19 knockout Raji cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% 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 antibody used was Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - HRP Anti-CD19 antibody [SP291] (C-terminal) (ab305999) at 1/1000 dilution

Lane 1:
Wild-type Raji at 20 µg

Lane 2:
Western blot - Human CD19 knockout Raji cell line (ab274913) at 20 µg

Lane 3:
Ramos at 20 µg

Lane 4:
MCF7 at 20 µg

Secondary

All lanes:
Goat anti-Mouse 680RD at 1/20000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 61 kDa

Observed band size: 80 kDa

Exposure time: 1s

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