Anti-CD20 Antibody [SY12-01]

ET1605-33

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Monkey
Applications:	WB, IHC-P, IF-Tissue, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 33 kDa
Clone number:	SY12-01

Description: CD20 is a leukocyte surface antigen consisting of four transmembrane regions and cytoplasmic N- and C-termini. The cytoplasmic domain of CD20 contains multiple phosphorylation sites, leading to additional isoforms. CD20 is expressed primarily on B cells but has also been detected on both normal and neoplastic T cells. CD20 functions as a calcium-permeable cation channel, and it is known to accelerate the G0 to G1 progression induced by IGF-1. CD20 is activated by the IGF-1 receptor via the alpha subunits of the heterotrimeric G proteins. Activation of CD20 significantly increases DNA synthesis and is thought to involve basic helix-loop-helix leucine zipper transcription factors.

Immunogen: Synthetic peptide within Human CD20 aa 248-297 / 297.

Positive control: Raji cell lysate, Ramos cell lysate, Daudi cell lysate, human tonsil tissue, human spleen tissue, B-cell lymphoma tissue, Raji.

Subcellular location: Cell membrane.

Database links: SwissProt: P11836 Human

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-P	1:200
IF-Tissue	1:50
IF-Cell	1:100
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

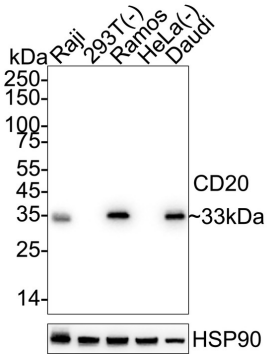

Fig1: Western blot analysis of CD20 on different lysates with Rabbit anti-CD20 antibody (ET1605-33) at 1/2,000 dilution.

Lane 1: Raji cell lysate
Lane 2: 293T cell lysate (negative)
Lane 3: Ramos cell lysate
Lane 4: HeLa cell lysate (negative)
Lane 5: Daudi cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 33 kDa
Observed band size: 33 kDa

Exposure time: 4 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1605-33) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

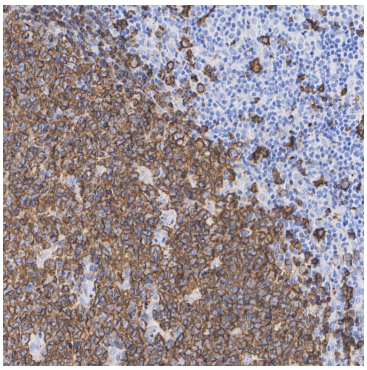

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD20 antibody (ET1605-33) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1605-33) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

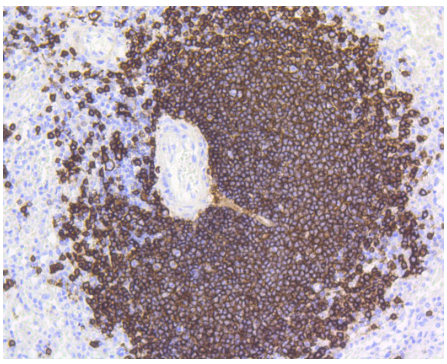

Fig3: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-CD20 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1605-33, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

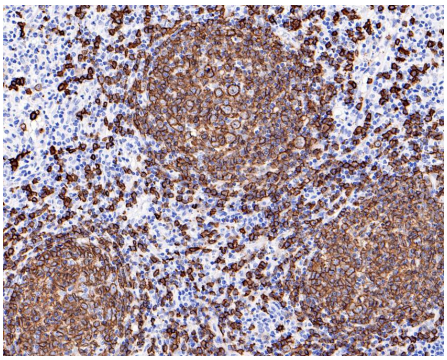

Fig4: Immunohistochemical analysis of paraffin-embedded B-cell lymphoma tissue using anti-CD20 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1605-33, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

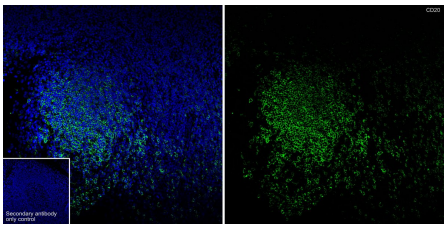

Fig5: Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling CD20 with Rabbit anti-CD20 antibody (ET1605-33) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1605-33, green) at 1/50 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

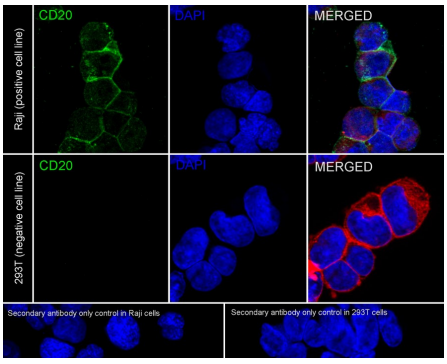

Fig6: Immunocytochemistry analysis of Raji (positive) and 293T (negative) labeling CD20 with Rabbit anti-CD20 antibody (ET1605-33) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD20 antibody (ET1605-33) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

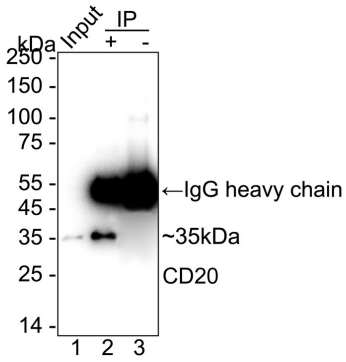

Fig7: CD20 was immunoprecipitated from 0.2 mg Raji cell lysate with ET1605-33 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1605-33 at 1/2,000 dilution. Mouse anti Rabbit IgG heavy chain (Fc) secondary antibody (M1003-7) at 1/10,000 dilution was used for 1 hour at room temperature.

Lane 1: Raji cell lysate (input)
Lane 2: ET1605-33 IP in Raji cell lysate
Lane 3: Rabbit IgG instead of ET1605-33 in Raji cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 10 seconds; ECL: K1801

Background References

1. Alhejaily A et al. Inactivation of the CDKN2A tumor-suppressor gene by deletion or methylation is common at diagnosis in follicular lymphoma and associated with poor clinical outcome. Clin Cancer Res 20:1676-86 (2014).
2. Xiang Z et al. Targeted Activation of Human V 9Vd2-T Cells Controls Epstein-Barr Virus-Induced B Cell Lymphoproliferative Disease. Cancer Cell 26:565-76 (2014).