

| DESCRIPTION | |
|---------------------------|---|
| Species Reactivity | Human |
| Specificity | Detects human CD20 in direct ELISAs. |
| Source | Recombinant Monoclonal Rabbit IgG Clone # 2709D |
| Purification | Protein A or G purified from cell culture supernatant |
| Immunogen | Human CD20 Peptide. The epitope recognized by rituximab Binder M, et al. (2006) 108:1975-8. doi: 10.1182/blood-2006-04-014639. Epub 2006 May 16. PMID: 16705086 |
| Formulation | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS. |

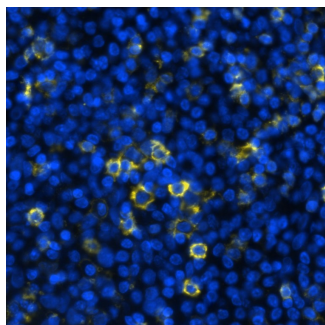
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

| | Recommended Concentration | Sample |
|-------------------------------------|-------------------------------|---|
| Western Blot | 1 µg/mL | Daudi human Burkitt's lymphoma cell line and Ramos human Burkitt's lymphoma cell line |
| Flow Cytometry | 0.25 µg/10 ⁶ cells | Human PBMC |
| Multiplex Immunofluorescence | 3 µg/mL | Immersion fixed paraffin embedded sections of human tonsil |
| Immunohistochemistry | 3-25 µg/mL | Immersion fixed paraffin-embedded sections of human tonsil |
| Simple Western | 25 µg/mL | Raji human Burkitt's lymphoma cell line |

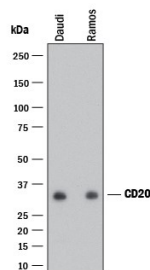
DATA

Multiplex Immunofluorescence



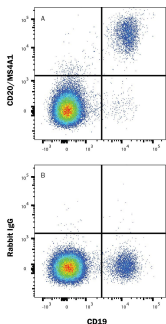
Detection of CD20 in Human Tonsil via Multiplex Immunofluorescence staining on COMET™ CD20 was detected in immersion fixed paraffin-embedded sections of human tonsil using Rabbit Anti-Human CD20 Monoclonal Antibody (Catalog # [MAB42252](#)) at 3µg/mL dilution at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # [DR647RB](#)) and counterstained with DAPI (blue; Lunaphore Catalog # [DR100](#)). Specific staining was localized to the membrane of B-cells. Protocol available in [COMET™ Panel Builder](#).

Western Blot



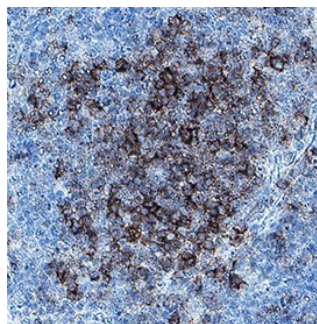
Detection of Human CD20 by Western Blot. Western blot shows lysates of Daudi human Burkitt's lymphoma cell line and Ramos human Burkitt's lymphoma cell line. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human CD20 Monoclonal Antibody (Catalog # [MAB42252](#)) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # [HAF008](#)). A specific band was detected for CD20 at approximately 33 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Flow Cytometry



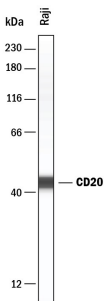
Detection of CD20 in Human PBMCs by Flow Cytometry. Human PBMCs were stained with (A) Rabbit Anti-Human CD20 Monoclonal Antibody (Catalog # [MAB42252](#)) or (B) Rabbit IgG Isotype Control Antibody (Catalog # [MAB1050](#)), followed by APC-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # [F0111](#)) and Mouse anti-Human CD19 PE-conjugated Monoclonal Antibody (Catalog # [FAB4867P](#)). Staining was performed using our Staining Membrane-associated Proteins protocol.

Immunohistochemistry



CD20 in Human Tonsil. CD20 was detected in immersion fixed paraffin-embedded sections of human tonsil using Rabbit Anti-Human CD20 Monoclonal Antibody (Catalog # [MAB42252](#)) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # [VC003](#)). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # [CTS013](#)). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human CD20 by Simple Western™. Simple Western lane view shows lysates of Raji human Burkitt's lymphoma cell line, loaded at 0.2 mg/mL. A specific band was detected for CD20 at approximately 42 kDa (as indicated) using 25 µg/mL of Rabbit Anti-Human CD20 Monoclonal Antibody (Catalog # [MAB42252](#)). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

| | |
|--------------------------------|---|
| Reconstitution | Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration. |
| Shipping | Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below. |
| Stability & Storage | <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution. |

BACKGROUND

CD20 is a 297 amino acid (aa) non-glycosylated type III membrane protein that is the founding member of the MS4A tetraspanin protein family. It is expressed on pre-B, naïve and mature B lymphocytes and B-cell lymphomas. CD20 is associated with lipid rafts upon crosslinking, and appears to play a role in regulation of ion influx. The one extracellular segment of 48 aa shows 65% aa sequence identity between human and mouse CD20.