Anti- Rituximab (Anti-Idiotype) Antibody
Recombinant Monoclonal Rabbit IgG Clone # 2260A
Catalog Number: MAB9630

DESCRIPTION
Specificity
Detects Rituximab in Western blots in direct ELISAs. 100% cross-reactivity with Tositumomab and Ibritumomab (95% or higher sequence identity with Rituximab) and no cross-reactivity with Cetuximab, Trastuzumab, Brentuximab, Gemtuzumab, Adalimumab, Solanezumab, Teprotumumab, and Aducanumab is observed.

Source
Recombinant Monoclonal Rabbit IgG Clone # 2260A

Purification
Protein A or G purified from cell culture supernatant

Immunogen
Full-length Rituximab

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

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Western Blot</td>
<td>0.5 µg/mL</td>
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<tr>
<td>Binding Inhibition</td>
<td>2.5 µg/mL</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>0.25 µg/10^6 cells</td>
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</tbody>
</table>

ELISA
Detects Rituximab, Ibritumomab and Tositumomab in direct ELISA.

DATA

Western Blot

Detection of Rituximab by Western Blot. Western blot shows Cetuximab, Rituximab, Brentuximab, and Gemtuzumab. PVDF membrane was probed with 0.5 µg/mL of Anti-Rituximab (Anti-Idiotype) Monoclonal Antibody (Catalog # MAB9630) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Rituximab at approximately 240 kDa (as indicated). This experiment was conducted under non-reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry

Detection of Anti-MS4A1/CD20 (Rituximab) on Human MS4A1/CD20 transfectants by flow cytometry. HEK293 human cell line transfected with (A) human MS4A1/CD20 or (B) irrelevant transfected and eGFP were stained with Human anti-Human MS4A1/CD20 (Rituximab) Monoclonal Antibody (Catalog # MAB9575), then Anti-Rituximab (Anti-Idiotype) (Catalog # MAB9630) followed by APC-conjugated anti-rabbit IgG secondary antibody (Catalog # F0111).

ELISA

Specificity of Anti-Rituximab (Anti-Idiotype) Antibody in ELISA Capture Assay. Biotinylated biosimilars were serially diluted and incubated with Anti-Rituximab (Anti-Idiotype) Antibody (Catalog # MAB9630), then Anti-Rituximab (Anti-Idiotype) (Catalog # MAB9630) followed by APC-conjugated anti-rabbit IgG secondary antibody (Catalog # F0111).

Binding Inhibition

Anti-MS4A1/CD20 (Rituximab) binding to MS4A1/CD20-transfected HEK293 Human Cell Line is Blocked by Anti-Rituximab (Anti-Idiotype) Antibody. In a functional flow-cytometry test, Biotinylated Anti-MS4A1/CD20 (Rituximab) (Catalog # FAB9575B) binds to HEK293 human embryonic kidney cell line transfected with human MS4A1/CD20 (black dotted line). Binding is completely blocked (orange histogram) by 0.25 µg/mL of Anti-Rituximab (Anti-Idiotype) Monoclonal Antibody (Catalog MAB9630), but not by Normal Rabbit IgG (Catalog # MAB1050) (blue line). Cells were stained with APC-conjugated Streptavidin (Catalog # F0050).
ELISA Specificity of Anti-Rituximab (Anti-Idiotype) Antibody in Direct ELISA. A panel of biosimilars was coated at 1 μg/mL in 96-well polystyrene plates. Anti-Rituximab (Anti-Idiotype) Antibody (Catalog # MAB9630, 333 ng/mL) detected Rituximab. 100% cross-reactivity with Tositumomab and Ibritumomab (95% or higher sequence identity with Rituximab) and no cross-reactivity with Cetuximab, Trastuzumab, Brentuximab, Gemtuzumab, Adalimumab, Solanezumab, Teprotumumab, and Aducanumab was observed.

ELISA Anti-Rituximab Bridging Assay. The anti-idiotype antibody (catalog number MAB9630) directed against Rituximab was coated at 4 μg/mL in a 96-well polystyrene plate to capture the biosimilar Rituximab. Biotinylated anti-Rituximab (Anti-Idiotype) Antibody was used at 0.5 μg/mL as the detection antibody before incubation with Streptavidin-HRP. Other biosimilars did not bridge the anti-idiotype antibody, showing specificity of the anti-Rituximab (Anti-Idiotype) antibody.

PREPARATION AND STORAGE
Reconstitution Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 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
The anti-Rituximab antibody is an anti-Idiotype antibody raised against and selected for the antigenic determinants of the variable region of Rituximab. The antibody has been tested against a broad range of other biosimilars to ensure specificity. This highly specialized anti-idiotypic antibody can be used in a variety of pharmacokinetic (PK) assay formats. In addition to Rituximab, the anti-Idiotype antibody also detects derivatives of Rituximab (Ibritumomab and Tositumumab).