BB515 Rat Anti-Mouse OX40 Ligand (CD252)

Product Information

Material Number: 565214
Alternate Name: Tnfsf4; OX40 ligand; OX40L; TXGP1; Txgp1l; gp34; Ath-1, Ath1, CD134L
Size: 50 µg
Concentration: 0.2 mg/ml
Clone: RM134L
Immunogen: Rat NRK-52E cell line transfected with mouse OX-40L gene (Txgp1l)
Isotype: Rat (SD) IgG2b, κ
Reactivity: QC Testing: Mouse
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The RM134L antibody reacts with CD252 (OX-40 Ligand, OX-40L), a member of the NGF/TNF superfamily which is present on antigen-presenting cells and activated B lymphocytes. OX-40L interacts with OX-40 Antigen (CD134) found predominantly on activated T cells. This ligand-receptor pair is grouped with pairs such as CD40-CD40L and CD80- or CD86-CD28, which contribute significantly to B-cell/T-cell interaction during the immune response. The OX-40L-OX-40 interaction is reciprocally costimulatory in that both T cells and B cells are activated in cross-linking. Stimulation via OX-40 Antigen increases the proliferative and IL-2 production responses of activated T cells, while stimulation via OX-40L enhances proliferation and Ig secretion by activated B cells. The RM134L mAb stains B cells activated for four days with anti-IgM plus anti-CD40 (Clone HM40-3) antibodies. An increased binding of OX-40-Ig fusion protein to mouse splenic B cells was observed when B cells were treated with lipopolysaccharide (LPS), suggesting that OX-40L expression is augmented in LPS-activated splenic B cells when compared to resting cells, but this observation could not be confirmed with the RM134L mAb. In addition, other studies with OX-40-Ig fusion protein detected OX-40L on CD4+ and CD8+ activated splenic T cells, but OX-40L was not detected on T cells with the RM134L mAb. Similar results have been reported by others. The RM134L mAb inhibits the binding of OX-40-Ig fusion protein to OX-40L transfectants and blocks the costimulatory activity of OX-40L.

The antibody was conjugated to BD Horizon BB515 which was developed exclusively by BD Biosciences. With an excitation max of 490 nm and an emission max of 515 nm, BD Horizon BB515 can be excited by the 488 nm laser and detected in a standard FITC set (e.g. 530/30-nm filter). This dye provides a much brighter alternative to FITC with less spillover into the PE detector.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry Routinely Tested

Flow cytometric analysis of mouse OX40 ligand (CD252) expression on stimulated mouse splenocytes. Mouse splenic leucocytes were stimulated (4 days) in culture with Purified NA/LE Hamster Anti-Mouse CD40 antibody (Cat. No. 553721) and Goat Anti-Mouse IgG1 (Jackson Immunoresearch 115-006-020). The cells were harvested, preincubated with Purified Rat Anti-Mouse CD16/CD32 antibody (Mouse BD Fc Block™) (Cat. No. 553141/553142), and then stained with either BD Horizon™ BB515 Rat IgG2b, κ Isotype Control (Cat. No. 564421, dashed line histogram) or BD Horizon BB515 Rat Anti-Mouse OX40 Ligand (CD252) antibody (Cat. No. 565214, solid line histogram). The fluorescence histogram showing OX40 ligand (CD252) expression (or Ig Isotype control staining) was derived from gated events with the forward and side light-scatter characteristics of viable splenocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ BB515 under optimum conditions and unconjugated antibody was removed.

Application Notes

Application

Flow cytometry Routinely Tested
Recommended Assay Procedure:
For optimal results, it is recommended to perform 2 washes after staining with antibodies. Cells may be prepared, stained with antibodies and washed twice with wash buffer per established protocols for immunofluorescence staining, prior to acquisition on a flow cytometer. Performing fewer than the recommended wash steps may lead to increased spread of the negative population.

For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. The BD Horizon Brilliant Stain Buffer was designed to minimize these interactions. More information can be found in the Technical Data Sheet of the BD Horizon Brilliant Stain Buffer (Cat. No. 563794/566349).

Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554656</td>
<td>Stain Buffer (FBS)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>554657</td>
<td>Stain Buffer (BSA)</td>
<td>500 mL</td>
<td>(none)</td>
</tr>
<tr>
<td>563794</td>
<td>Brilliant Stain Buffer</td>
<td>100 Tests</td>
<td>(none)</td>
</tr>
<tr>
<td>553721</td>
<td>Purified NA/LE Hamster Anti-Mouse CD40</td>
<td>0.5 mg</td>
<td>HM40-3</td>
</tr>
<tr>
<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.1 mg</td>
<td>2.4G2</td>
</tr>
<tr>
<td>553142</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.5 mg</td>
<td>2.4G2</td>
</tr>
<tr>
<td>564421</td>
<td>BB515 Rat IgG2b, κ Isotype Control</td>
<td>0.1 mg</td>
<td>R35-38</td>
</tr>
<tr>
<td>566349</td>
<td>Brilliant Stain Buffer</td>
<td>1000 Tests</td>
<td>(none)</td>
</tr>
</tbody>
</table>

Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. The manufacture, use, sale, offer for sale, or import of this product is subject to one or more patents or pending applications. This product, and only in the amount purchased by buyer, may be used solely for buyer’s own internal research, in a manner consistent with the accompanying product literature. No other right to use, sell or otherwise transfer (a) this product, or (b) its components is hereby granted expressly, by implication or by estoppel. Diagnostic uses require a separate license.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.

References