BD Pharmingen™ Technical Data Sheet

FITC Mouse Anti-Rat IgG2a

Product Information
Material Number: 553896
Size: 0.5 mg
Concentration: 0.5 mg/ml
Clone: RG7/1.30
Immunogen: Rat Pooled IgG
Isotype: Mouse (SJL) IgG2b, κ
Reactivity: QC Testing: Rat
Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description
The RG7/1.30 antibody reacts specifically with the Fc region of rat IgG2a. It does not react with other Ig isotypes.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Preparation and Storage
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

<table>
<thead>
<tr>
<th>Application</th>
<th>Flow cytometry</th>
<th>Routinely Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular staining (flow cytometry)</td>
<td>Tested During Development</td>
<td></td>
</tr>
</tbody>
</table>

Recommended Assay Procedure:

Immunofluorescent Staining of Intracellular Immunoglobulin (Ig) Protocol

1. Prepare a single-cell suspension and determine cell number.
2. Suspend cells in staining buffer (PBS + 2% FBS + 0.1% Sodium Azide) at 2 x 10^6 cells/ml and transfer to U-bottom microwell plates in 50 μl/well for immunofluorescent staining.

Note: The BD Pharmingen™ Stain Buffer with FBS (Cat. No. 554656) is effective for use as a staining buffer in this protocol.
3. Block Fc receptors by adding 0.2 µg of purified 2.4G2 antibody (Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2) (Cat. No. 553141/553142) in 50 µl of staining buffer to each well.
4. Incubate 5 minutes on ice.
5. Add 200 µl of staining buffer/well and resuspend cells. Centrifuge at 250 x g for 5 minutes and aspirate supernatant.
6. Block surface Ig with purified RG7/1.30 mAb (Cat. No. 553893) by adding 1.0 µg per sample in 50 µl of staining buffer/well.

Note: Surface markers may be stained during this step as described in the "Immunofluorescent Staining of Mouse and Rat Leukocytes for Flow Cytometry" in the Technical Protocols section of our website at www.bdbiosciences.com/pharmentis/protocols/Mouse_and_Rat_Leukocytes.shtml

7. Incubate 15 minutes on ice.
8. Wash 2x as described in Step 5.
9. Resuspend cells in 100 µl of BD Cytofix/Cytoperm™ intracellular staining buffer (BD Cytofix/Cytoperm Kit, Cat. No. 554714) per well.
10. Incubate 30 minutes at room temperature.
11. Wash 2x with 200 µl of 1 x Perm/Wash buffer (provided in the BD Cytofix/Cytoperm Kit) per well. Centrifuge at 250 x g for 5 minutes and aspirate supernatant between washes.
12. Stain intracellular Ig by adding ≤ 1 µg of FITC-conjugated RG7/1.30 mAb in 50 µl of 1 x Perm/Wash buffer/well.

Note: Other antibodies recommended for staining of intracellular markers may be added during this step as described in Step 12.

13. Incubate for 30 minutes at room temperature.
14. Wash 2x as described in Step 11.
15. Resuspend and transfer samples in 100 µl of staining buffer to tubes appropriate for analysis with a flow cytometer. Bring volume in each tube to 400 µl with staining buffer.
16. Analyze samples on a flow cytometer.

**Suggested Companion Products**

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554714</td>
<td>BD Cytofix/Cytoperm Fixation/Permeabilization Kit</td>
<td>250 tests</td>
<td>(none)</td>
</tr>
<tr>
<td>555057</td>
<td>FITC Mouse IgG2b, κ Isotype Control</td>
<td>0.1 mg</td>
<td>27-35</td>
</tr>
</tbody>
</table>

**Product Notices**

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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.

**References**