PerCP-Cy™5.5 Rat Anti-Mouse TNF

Product Information

Material Number: 560659

Size: 50 µg

Concentration: 0.2 mg/ml

Clone: MP6-XT22

Immunogen: Recombinant mouse TNF

Isotype: Rat IgG1

Reactivity: QC Testing: Mouse

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MP6-XT22 antibody reacts with mouse tumor necrosis factor (TNF, also known as TNF-α). The immunogen used to generate this hybridoma was recombinant mouse TNF.

Flow cytometric analysis for TNF in activated mouse splenocytes. Mouse Intracellular Cytokine-1 positive control cells (MiCK-1) offered by BD Biosciences as MN 554652, are activated mouse splenocytes prepared in the presence of a protein transport inhibitor. Fixed and permeabilized MiCK-1 cells were stained either with a PerCP-Cy™5.5 Rat IgG1, κ isotype control (left panel) or with the PerCP-Cy™5.5 Rat Anti-Mouse TNF antibody (right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry 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 PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

| Intracellular staining (flow cytometry) | Routinely Tested |

Recommended Assay Procedure:

Flow cytometry: The MP6-XT22 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate TNF producing cells within mixed cell populations. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant mouse TNF (Cat. No. 554589) or (2) unlabeled MP6-XT22 antibody (Cat. No. 554416), prior to staining.

Cell Preparation: Investigators not wishing to utilize MiCK-1 cells may alternatively prepare mouse splenocytes (e.g BALB/c) stimulated for 4-6 hours with PMA (5 ng/mL, Sigma-Aldrich Cat. No. P-8139) and ionomycin (500 ng, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug™ MN 555029). Investigators are advised to fix and permeabilize the cells prior to staining.
Suggested Companion Products

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>560537</td>
<td>PerCP-Cy™5.5 Rat IgG1, κ Isotype Control</td>
<td>0.1 mg</td>
<td>R3-34</td>
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<tr>
<td>554652</td>
<td>MiCK-1 Mouse Cytokine Positive Control Cells</td>
<td>1.0 ml</td>
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<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>
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<tr>
<td>554416</td>
<td>Purified Rat Anti-Mouse TNF</td>
<td>0.1 mg</td>
<td>MP6-XT22</td>
</tr>
<tr>
<td>555028</td>
<td>BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)</td>
<td>250 tests</td>
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<tr>
<td>555029</td>
<td>Protein Transport Inhibitor (Containing Brefeldin A)</td>
<td>1.0 ml</td>
<td>(none)</td>
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<tr>
<td>554589</td>
<td>Recombinant Mouse TNF</td>
<td>10 µg</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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
4. This PerCP-conjugated product is sold under license to the following patent: US Patent No. 4,876,190.
5. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
6. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121. Any money paid for the material will be refunded.
7. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
8. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
9. 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.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

References


Drew PD, Chavis JA. Female sex steroids: effects upon microglial cell activation. *J Neuroimmunol*. 2000; 115(1-2):77-85. (Biology)


