

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human/mouse ROR γ t/RORC2/NR1F3: Supplied as 10 μ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 600380

Isotype: mouse IgG_{2b}

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

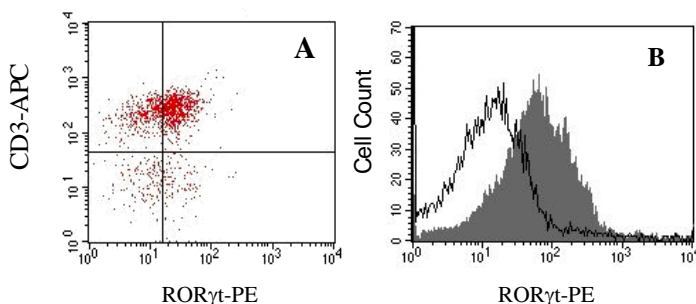
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing ROR γ t/RORC2 within a population and qualitatively determine the density of intracellular ROR γ t/RORC2 by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with ROR γ t peptide (aa 1 - 10; Accession # P51449). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Intracellular expression of ROR γ t/RORC2 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.



A) Human PBMCs were treated with PMA/Ca²⁺ ionomycin, LPS, and recombinant human IL-23. Cells were co-stained with APC-conjugated anti-human CD3 (Catalog # FAB100A) and PE-conjugated anti-human/mouse ROR γ t/RORC2/NR1F3 (Catalog # IC6006P).
B) CD4⁺/CD8⁺ mouse thymocytes were stained with PE-conjugated anti-human/mouse ROR γ t/RORC2/NR1F3 (Catalog # IC6006P).

Background Information

Retinoic acid-related Orphan Receptor gamma (ROR γ , TOR, RORC; NR1F3) is a member of the orphan nuclear receptor family. ROR γ is expressed in the muscle, thymus, testis, pancreas, prostate, heart, and liver. ROR γ plays a role in thymocyte development and homeostasis. RORs bind to DNA as monomers on half-site elements with 5' A/T-rich extensions. An N-terminal isoform of ROR γ , ROR γ t, has been shown to be specifically expressed in the thymus. **By direct ELISA, clone 600380 has been shown to specifically recognize the ROR γ t/RORC2 isoform, and not RORC.**

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see [Reagents Not Provided](#)).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer's staining procedure.
3. Up to 1 x 10⁶ cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 μ L of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2b} antibody. This procedure may need to be modified, depending on the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.