Monoclonal Anti-human RUNX3/CBFA3-APC

Catalog Number: IC3765A
Lot Number: ABKO01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human RUNX3/CBFA3: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 527327

Isotype: mouse IgG_{2A}

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

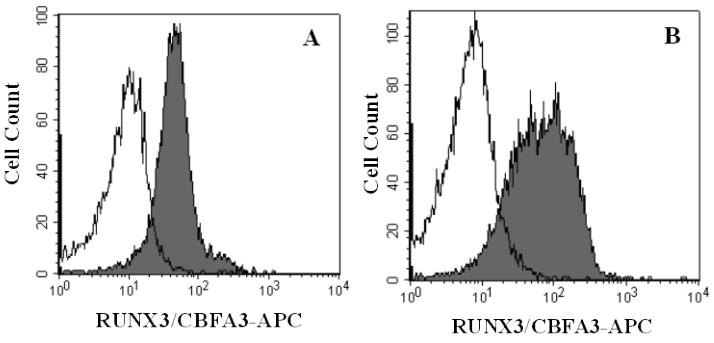
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing RUNX3/CBFA3 within a population and qualitatively determine the density of intracellular RUNX3/CBFA3 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 purified, *E. coli*-derived recombinant human RUNX3 (rhRUNX3; aa 186 - 415; Accession # Q13761). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Intracellular expression of RUNX3/CBFA3 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



A) PBMC lymphocytes and B) PBMC lymphocytes activated with PMA were stained with APC-conjugated anti-human RUNX3/CBFA3 (Catalog # IC3765A, filled histogram) or APC-conjugated mouse isotype control (Catalog # IC003A, open histogram).

Background Information

RUNX3, also called CBFA3, AML-2 or PEBP2-αC, is a member of the Runt domain family of nuclear transcriptional regulators. All of the RUNX proteins form dimers with CBF-β. The runt domain (amino acids 54 - 186) is required for DNA binding, while a Pro/Ser/Thr-rich region (amino acids 191 - 415) transcriptionally activates target genes. Isoform 2 has an alternate 19 amino acid (aa) sequence in place of the N-terminal 5 aa of isoform 1. The 415 aa Human RUNX3 shows 91% aa identity with mouse or rat RUNX3. RUNX3 is necessary for growth control of gastric epithelium, neurogenesis of dorsal root ganglia, and T cell differentiation. RUNX3 expression is frequently mutated in tumors and appears to be silenced by methylation.

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. Resuspend up to 1 x 10^6 cells in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubate 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 μL 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 APC-labeled mouse IgG_{2A} 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.

R&D Systems Inc.
1-800-343-7475