

DESCRIPTION

| | |
|---------------------------|---|
| Species Reactivity | Human/Mouse |
| Specificity | Detects human and mouse RUNX3/CBFA3 in Western blots. |
| Source | Monoclonal Mouse IgG _{2A} Clone # 527327 |
| Purification | Protein A or G purified from hybridoma culture supernatant |
| Immunogen | <i>E. coli</i> -derived recombinant human RUNX3/CBFA3 Lys186-Tyr415 Accession # Q13761 |
| Formulation | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS. |

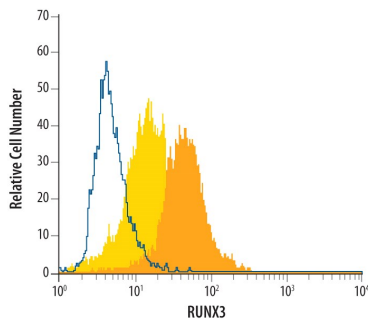
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

| | Recommended Concentration | Sample |
|---|--|-----------|
| Western Blot | 1 µg/mL | See Below |
| Immunocytochemistry | 8-25 µg/mL | See Below |
| Intracellular Staining by Flow Cytometry | 2.5 µg/10 ⁶ cells | See Below |
| Simple Western | 10 µg/mL | See Below |
| CyTOF-ready | Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation. | |

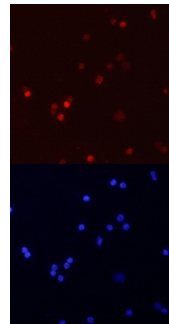
DATA

Intracellular Staining by Flow Cytometry



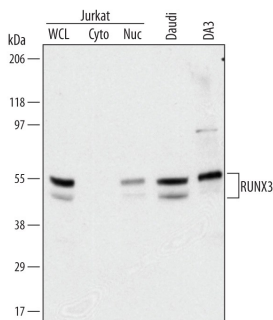
Detection of RUNX3/CBFA3 in Human PBMC by Flow Cytometry. Human PBMC unstimulated (light orange filled histogram) or treated with 50ng/mL PMA (dark orange filled histogram) were stained with Mouse Anti-Human/Mouse RUNX3/CBFA3 Monoclonal Antibody (Catalog # MAB3765) or isotype control antibody (Catalog # MAB003, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG F(ab)₂ Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

Immunocytochemistry



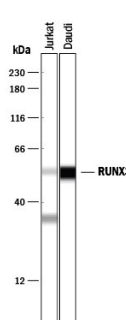
RUNX3/CBFA3 in Human PBMCs. RUNX3/CBFA3 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Mouse Anti-Human/Mouse RUNX3/CBFA3 Monoclonal Antibody (Catalog # MAB3765) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 567-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

Western Blot



Detection of Human and Mouse RUNX3/CBFA3 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, Daudi human Burkitt's lymphoma cell line, and DA3 mouse myeloma cell line. Gels were loaded with 30 µg of whole cell lysate (WCL), 20 µg of cytoplasmic (Cyto), and 10 µg of nuclear extracts (Nuc). PVDF Membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse RUNX3/CBFA3 Monoclonal Antibody (Catalog # MAB3765) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Specific bands were detected for RUNX3/CBFA3 at approximately 48 and 52 kDa (as indicated). This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

Simple Western



Detection of Human RUNX3/CBFA3 by Simple Western™. Simple Western lane view shows lysates of Jurkat human acute T cell leukemia cell line and Daudi human Burkitt's lymphoma cell line, loaded at 0.5 mg/mL. A specific band was detected for RUNX3/CBFA3 at approximately 54 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human/Mouse RUNX3/CBFA3 Monoclonal Antibody (Catalog # MAB3765). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution Reconstitute at 0.5 mg/mL in sterile PBS.

Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

RUNX3, also called CBFA3, AML-2 or PEBP2- α C, 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 (aa 54-186) is required for DNA binding, while a pro/ser/thr-rich region (aa 191-415) transcriptionally activates target genes. Isoform 2 has an alternate 19 aa in place of the N-terminal 5 aa of isoform 1. The 415 aa Human RUNX3 shares 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.