

## Reagents Provided

**Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse DMBT1:**  
Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 548031

**Isotype:** rat IgG<sub>2b</sub>

## Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

## Storage

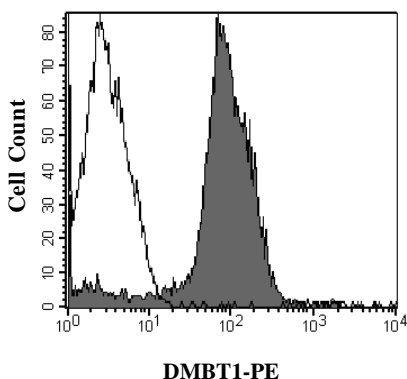
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing DMBT1 within a population and qualitatively determine the density of DMBT1 on cell surfaces 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 mouse DMBT1 (rmDMBT1; aa 215 - 420) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of DMBT1 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.



CMT-93 cells were stained with PE-conjugated anti-mouse DMBT1 (Catalog # FAB5915P, filled histogram) or isotype control (Catalog # IC013P, open histogram).

## Background Information

DMBT1 (Deleted in malignant brain tumor-1; also MUCCLIN, Hensin, CRP-Ductin and gp300/350) is a 230 - 350 kDa member of the DMBT1 family of molecules. It is expressed by select cell types including macrophages, intestinal epithelium, and pancreatic acinar cells. DMBT1 is both a transmembrane (TM) and secreted protein, and possesses multiple activities that may be predicated on multimerization. It drives epithelial differentiation, binds Gm+ and Gm- bacteria, and associates with cytoskeletal actin. Mouse DMBT1 is a type I TM glycoprotein that is 2085 amino acids (aa) in length. Its extracellular region contains eight SRCR repeats (aa 31 - 980, plus 1510 - 1610), five CUB domains (aa 1023 - 1490, plus 1633 - 1742), and a ZP segment (aa 1751 - 1999). Multiple splice variants exist. There are deletions of aa 584 - 733, 397 - 534, 593 - 731 and 2032 - 2085 (within the TM segment), plus an insertion of 11 aa after Asp29. Over aa 215 - 420, mouse DMBT1 shares 75% aa identity with human DMBT1.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using CMT-93 cells.

- Cells may be Fc-blocked with 1 µg of mouse IgG/10<sup>5</sup> cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to 1 - 2.5 x 10<sup>5</sup> cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled rat IgG<sub>2b</sub> antibody. This procedure may need to be modified depending upon 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.