

## Reagents Provided

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

**Clone #:** 323519

**Isotype:** mouse IgG<sub>1</sub>

## Reagents Not Provided

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

## Storage

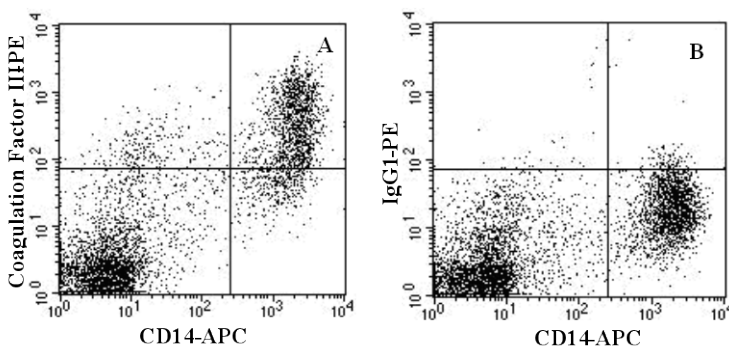
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing Coagulation Factor III/TF within a population and qualitatively determine the density of Coagulation Factor III/TF 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 NS0-derived recombinant human TF extracellular domain (rhTF; aa 34-251; Accession # P13726). 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 Coagulation Factor III/TF 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.



Human PBMC were stained with APC-conjugated anti-human CD14 (Catalog # FAB3832A) and either A) PE-conjugated anti-human Coagulation Factor III/TF (Catalog # FAB23391P) or B) isotype control (Catalog # IC002P).

## Background Information

Coagulation factor III/tissue factor (TF), also known as thromboplastin and CD142, is an integral membrane protein found in a variety of cell types that functions as a protein co-factor/receptor of Coagulation factor VII in the extrinsic pathway of blood clotting. Synthesized as a 295 amino acid (aa) precursor, TF consists of a signal peptide (aa 1-32) and the mature chain (aa 33-295). As a type I membrane protein, it contains a transmembrane region (aa 252-274) and a cytoplasmic tail (aa 275-295). The amino acid sequence of the human ectodomain is 76%, 75%, 60%, and 59% identical to that of dog, bovine/porcine/rabbit, mouse, and rat, respectively.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using human PBMCs.

- Cells may be Fc-blocked with 1 µg of human 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 up to 1 x 10<sup>6</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 Human Lyse Buffer (Catalog # FC002).
- 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 mouse IgG<sub>1</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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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