

## Reagents Provided

**Allophycocyanin (APC)-conjugated rat monoclonal anti-mouse CEACAM-1/CD66a:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

**Clone #:** 723629

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

## Reagents Not Provided

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

## Storage

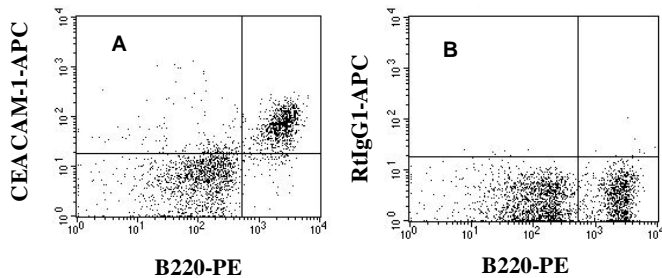
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing CEACAM-1/CD66a within a population and qualitatively determine the density of CEACAM-1/CD66a 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 rat immunized with purified NS0-derived recombinant mouse CEACAM-1/CD66a (aa 35-428; Accession # P31809). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of CEACAM-1/CD66a 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.



Mouse splenocytes were stained with PE-conjugated anti-mouse B220 (Catalog # FAB1217P) and A) APC-conjugated anti-mouse CEACAM-1/CD66a (Catalog # FAB6480A) or B) APC-conjugated isotype control (Catalog # IC005A).

## Background Information

CEACAM-1 (Carcinoembryonic antigen-related cell adhesion molecule 1; also known as BGP1, CD66a, and MHVR1) is a 110-120 kDa member of the CEACAM subfamily and the CEA family of proteins. It has a wide expression pattern, being found on neutrophils, dendritic cells, endothelial cells, colonic epithelium, and hepatocytes. It mediates cell adhesion, and appears to regulate insulin levels and signaling by interacting with the insulin receptor. It also demonstrates pro-angiogenic effects by inducing endothelial cells to proliferate and form capillary-like tubules. Finally, CEACAM-1 is a known receptor for mouse hepatitis virus. Mature mouse CEACAM-1 is a 487 amino acid (aa) type I transmembrane glycoprotein. It contains a 394 aa extracellular region (aa 35-428) that shows one V-type (aa 35-142) and three C2-type (aa 147-411) Ig-like domains, plus a 74 aa cytoplasmic domain. Three alternate splice forms exist. One contains a four aa substitution for aa 455-521, a second shows a Gln substitution for aa 142-322, and a third possesses a combination of the first two splice patterns. CEACAM-1 forms homodimers. Over aa 35-428, mouse CEACAM-1 shares 56% and 70% aa identity with human and rat CEACAM-1, respectively.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

- 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 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 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 APC-labeled rat 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.