

# Monoclonal Anti-human CEACAM-6-Allophycocyanin

Catalog Number: FAB3934A

Lot Number: AAWC01

100 Tests

## Reagents Provided

**Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CEACAM-6:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 439424

Isotype: mouse IgG<sub>2A</sub>

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

## Storage

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

## Intended Use

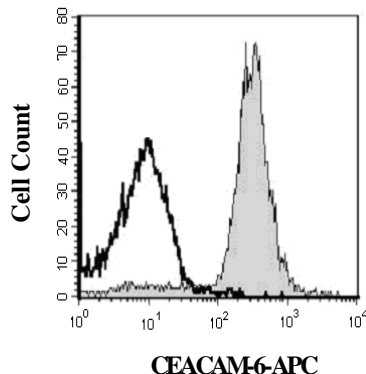
Designed to quantitatively determine the percentage of cells bearing CEACAM-6 within a population and qualitatively determine the density of CEACAM-6 on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing CEACAM-6. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing CEACAM-6 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of CEACAM-6. Cell surface expression of CEACAM-6 is determined by flow cytometry using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

## Reagent Preparation

**Allophycocyanin-conjugated mouse anti-human CEACAM-6:** Use as is; no preparation necessary.



Human granulocytes were stained with APC-conjugated anti-human CEACAM-6 (Catalog # FAB3934A, filled histogram) or isotype control (Catalog # IC003A, open histogram).

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA) to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10<sup>6</sup> cells/mL and 25 µL of cells (1 x 10<sup>5</sup>) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

- 1) Cells should be Fc-blocked by treatment with 1 µg of human IgG/10<sup>5</sup> cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
- 2) Transfer 25 µL of the Fc-blocked cells (1 x 10<sup>5</sup> cells) or 50 µL of packed whole blood to a 5 mL tube.
- 3) Add 10 µL of APC-conjugated CEACAM-6 reagent.
- 4) Incubate for 30 - 45 minutes at 2° - 8° C.
- 5) Following this incubation, remove unreacted CEACAM-6 reagent by washing the cells twice in 4 mL of the same PBS buffer. (*Note: Whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000.*)
- 6) Finally, resuspend the cells in 200 - 400 µL of PBS buffer for analysis by flow cytometry.
- 7) As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG<sub>2A</sub> antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Background Information**

CEACAM-6, previously called nonspecific cross-reacting antigen (NCA) or CD66c, is a highly glycosylated 90 kDa, 286 amino acid (aa), GPI-linked membrane protein of the human CEACAM family within the immunoglobulin superfamily. CEACAM-6 is an intercellular adhesion molecule, forming homotypic and heterotypic bonds with CEACAM-1, -5 and -8. Mature human CEACAM-6 shares 84% and 85% aa identity with human CEACAM-1 and -5 (CEA) respectively. It is expressed in azurophilic granules in cells of the granulocyte lineage, and in epithelia of various organs.

**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.