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## 1. Description

<b>Components</b>	1 mL monoclonal Anti-CRTAM antibodies, human conjugated to various dyes.
	PE 130-096-156
	APC 130-096-158
<b>Clone</b>	Cr24.1 (isotype: mouse IgG2a).
<b>Capacity</b>	100 tests or up to $2 \times 10^8$ total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

The anti-CRTAM antibody reacts with the human class I-restricted T cell-associated molecule. CRTAM is expressed on activated class I MHC restricted T cells, including CD8<sup>+</sup> T cells and NKT cells. It binds nectin-like protein 2 (Nect2). The binding of CRTAM and Nect2 triggers cytotoxicity of NK cells and IFN- $\gamma$  secretion by CD8<sup>+</sup> T cells.

### 1.2 Applications

- Identification and enumeration of CRTAM<sup>+</sup> cells by flow cytometry or fluorescence microscopy.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-CRTAM conjugates is **1:11 for up to  $2 \times 10^6$  cells/100  $\mu$ L** of buffer for labeling of cells and analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

### 1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
  - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD8-VioBlue<sup>®</sup> (# 130-094-152). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Mouse IgG2a isotype control antibodies conjugated to, e.g., PE (# 130-091-835). For more information about isotype control antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $2 \times 10^6$  nucleated cells. When working with fewer than  $2 \times 10^6$  cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for  $4 \times 10^6$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300 $\times$ g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $2 \times 10^6$  nucleated cells per 90  $\mu$ L of buffer.
4. Add 10  $\mu$ L of FcR Blocking Reagent.
5. Add 10  $\mu$ L of the Anti-CRTAM antibody.
6. Mix well and incubate for 40 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

7. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

### 3. Examples of immunofluorescent staining with Anti-CRTAM antibodies

Human peripheral blood mononuclear cells (PBMCs) were incubated with or without PMA (10 ng/mL) and ionomycin (1 µg/mL) for four hours at 37 °C and 5% CO<sub>2</sub>. Cells were then stained with Anti-CRTAM antibodies conjugated to PE (A) or APC (B) as well as with CD8-VioBlue (# 130-094-152) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

#### Warnings

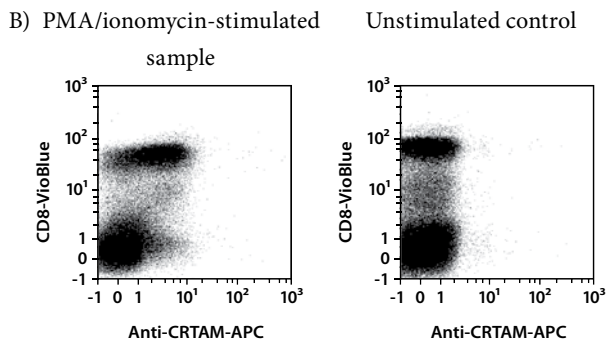
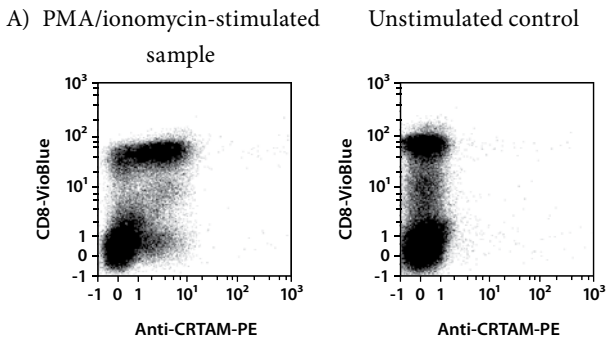
Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

#### Warranty

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### 4. Reference

1. Boles, K.S. *et al.* (2005) The tumor suppressor TSLC1/NECL-2 triggers NK-cell and CD8<sup>+</sup> T-cell responses through the cell-surface receptor CRTAM. *Blood* 106: 779–786.