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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.

1. Description

This product is for research use only.

| | |
|-----------------------|--|
| Components | 1 mL monoclonal Anti-KLRG1 antibodies, mouse conjugated to: |
| | PE 130-096-858 |
| | APC 130-096-847 |
| | Biotin 130-096-850 |
| Clone | 2F1 (isotype: hamster IgG1). |
| Capacity | 100 tests or up to 10 ⁹ total cells. |
| Product format | Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| Storage | Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. |

1.1 Background information

- Antigen: KLRG1
- Expression patterns: The monoclonal antibody 2F1 reacts with killer cell lectin-like receptor G1 (KLRG1), a homodimeric type II transmembrane glycoprotein of 30-38 kDa subunits. The protein is the mouse homolog of the rat mast cell function-associated antigen (MAFA) and is expressed on a subset of NK cells and T cells. Several members of the cadherin family can interact with KLRG1 leading to inhibition of NK cell and T cell effector functions. KLRG1 is considered as a marker for identification of terminal differentiated NK cells and T cells with reduced proliferative and effector function abilities.

1.2 Applications

- Identification and enumeration of KLRG1⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-KLRG1 conjugates is **1:11 for up to 10⁷ cells/100 μL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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[®] BSA Stock Solution (#130-091-376) 1:20 with autoMACS[®] 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). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (#130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (#130-090-756) as secondary antibody reagent in combination with Anti-KLRG1-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (#130-093-233) for flow cytometric exclusion of dead cells without fixation.

2. General protocol for immunofluorescent staining

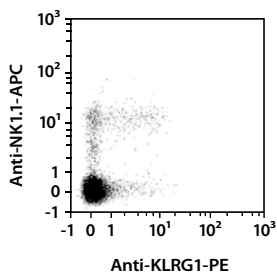
Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 μL of buffer.
4. Add 10 μL of the Anti-KLRG1 antibody.
5. Mix well and incubate for 10 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.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. (Optional) If Anti-KLRG1-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with Anti-KLRG1 antibodies

C57Bl/6 splenocytes were stained with Anti-KLRG1 antibodies conjugated to PE as well as with Anti-NK1.1-APC, mouse (# 130-095-869) 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.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

All protocols and data sheets are available at www.miltenyibiotec.com.

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