

# Anti-FR4 antibodies

## mouse

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### 1. Description

<b>Components</b>	<p>1 mL monoclonal Anti-FR4 antibodies, mouse conjugated to</p> <p>PE 130-095-243</p> <p>APC 130-095-239</p> <p>Biotin 130-095-246</p> <p>or</p> <p>0.5 mL monoclonal Anti-FR4 antibodies, mouse pure – functional grade 130-095-244</p>
<b>Clone</b>	TH6 (isotype: rat IgG2b).
<b>Capacity</b>	<p>100 tests or up to 10<sup>9</sup> total cells.</p> <p>The functional grade antibody is supplied at a concentration of 2 mg/mL.</p>
<b>Product format</b>	<p>Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.</p> <p>Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/μg of protein.</p> <p><i>The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.</i></p>
<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

Folate Receptor 4 (FR4), a subtype of the receptor for the vitamin folic acid, is described to be exclusively expressed in lymphoid tissue. It shows a constitutive and high expression on the surface of mouse natural CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. FR4 can be used for the detection of regulatory T cells in combination with the markers CD25 or FoxP3 as well as to discriminate regulatory T cells from effector T cells, memory T cells, and naive T cells.<sup>1</sup>

The Anti-FR4 pure – functional grade antibody is reported to abrogate regulatory T cell function *in vivo*.

#### 1.2 Applications

- Identification and enumeration of FR4<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse regulatory T cells can be isolated by using the CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit, mouse (# 130-091-041).
- *In vivo* depletion of regulatory T cells.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-FR4 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 μL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells. For optimal results, cells must be stained prior to fixation with formaldehyde or with the FoxP3 Staining Buffer Set (# 130-093-142).

#### 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). BSA can be replaced by other proteins such as mouse serum albumin, mouse 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, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-FR4-Biotin.
- (Optional) Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD4-FITC (# 130-091-608) and CD25-PE (# 130-091-013) or CD25-APC (# 130-093-734) and Anti-FoxP3-PE (# 130-093-014) or Anti-FoxP3-APC (# 130-093-013). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (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.

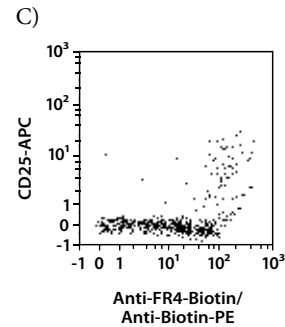
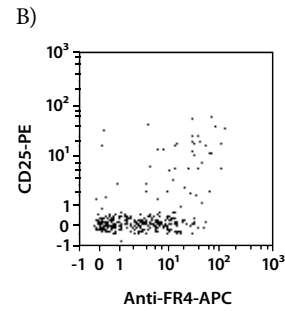
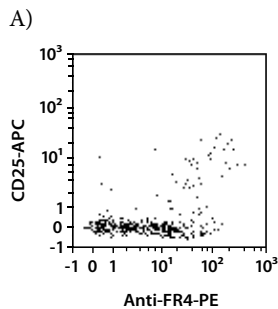
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  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  $2 \times 10^7$  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  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  FcR Blocking Reagent, mouse.
5. Add 10  $\mu\text{L}$  of the Anti-FR4 antibody.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
8. (Optional) If Anti-FR4-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of anti-biotin antibody, and continue as described in steps 6 and 7.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

## 3. Examples of immunofluorescent staining with Anti-FR4 antibodies

Mouse splenocytes were stained with Anti-FR4 antibodies conjugated to PE (A) or APC (B) as well as with CD25-APC (# 130-093-734) or CD25-PE (# 130-091-013) and CD4-FITC (# 130-091-608) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-FR4-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD25-APC and CD4-FITC. Gating was performed according to CD4 expression and side scatter properties. Cell debris and dead cells were excluded from the analysis based on propidium iodide fluorescence.



## 4. Reference

1. Yamaguchi, T. *et al.* (2007) Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. *Immunity* 27: 145–159.

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

### 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.

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