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1. Description

Components	1 mL monoclonal CD137 antibodies, mouse conjugated to various dyes.
	FITC 130-097-112
	PE 130-097-122
	APC 130-097-106
	Biotin 130-097-116
	or
	0.5 mL monoclonal CD137 antibodies, mouse pure – functional grade 130-097-139
Clone	17B5-1H1 (isotype: hamster IgG2).
Capacity	100 tests or up to 10^9 total cells. The functional grade antibody is supplied at a concentration of 1000 µg/mL.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide. 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. <i>The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.</i>
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

Clone 17B5 reacts with mouse CD137 also known as 4-1BB or TNFRSF9, a member of the TNFR superfamily. It is expressed on the cell surface of T cells upon activation. The interaction of CD137 with its ligand, 4-1BBL, on APCs delivers a bi-directional costimulatory signal. CD137 engagement is shown to be required for CD8⁺ T cell survival and memory formation. The monoclonal antibody 17B5 is reported to have strong antagonistic effect in *in vitro* functional assays.

1.2 Applications

- Identification and enumeration of CD137⁺ cells by flow cytometry or fluorescence microscopy.
- The CD137 pure – functional grade antibody is suited for functional assays, for example, blocking of receptor-ligand binding.]

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD137 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyd-fixed cells.

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 Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) 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 CD137-Biotin.
- (Optional) CD8a-FITC (# 130-091-605) or CD8a-APC (# 130-091-606). For more information about antibodies refer to 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

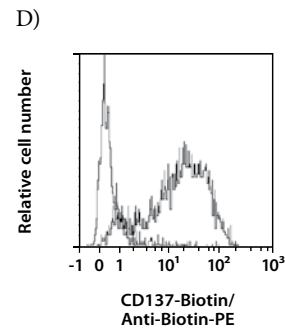
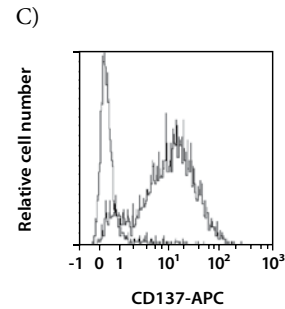
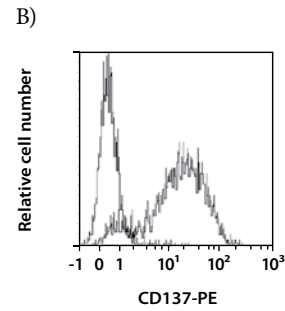
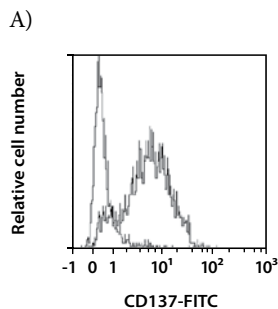
▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.

2. Centrifuge cell suspension at 300×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 CD137 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 CD137-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL 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. Examples of immunofluorescent staining with CD137 antibodies

Mouse spleen cells were stimulated with plate-bound CD3 and soluble CD28 antibodies for 3 days or were left untreated. Then cells were stained with CD137 antibodies conjugated to FITC (A), PE (B), APC (C) and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cells labeled with CD137-Biotin (D) were stained with Anti-Biotin-PE (# 130-090-756). 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.

All protocols and data sheets are available at 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.

Warranty

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