

# CD137 antibodies

## human

CD137-PE	130-093-475
CD137-APC	130-094-821
CD137-Biotin	130-094-824

### Contents

1. Description
  - 1.1 Background information
  - 1.2 Applications
  - 1.3 Recommended antibody dilution
  - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
  - 2.1 Protocol for *in vitro* stimulation for induction of CD137 expression
  - 2.2 Immunofluorescent staining
3. Examples of immunofluorescent staining with CD137 antibodies
4. References
5. Appendix

### 1. Description

<b>Components</b>	1 mL CD137 antibodies, human: monoclonal CD137 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
<b>Clone</b>	4B4-1 (isotype: mouse IgG1).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> 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.

Cross-reactivity: The CD137 antibody has been tested to react with rhesus monkey (*Macaca mulatta*) cells.

#### 1.1 Background information

The activation-induced antigen CD137 (4-1BB) is a 30 kDa glycoprotein of the tumor necrosis factor (TNF) receptor superfamily. It is mainly expressed on activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, activated B cells, and natural killer cells, but can also be found on resting monocytes and dendritic cells.

As a costimulatory molecule, CD137 is involved in the activation and survival of CD4, CD8, and NK cells. Its engagement enhances expansion of T cells and activates them to secrete cytokines.

CD137 has been described to be a suitable marker for antigen-specific activation of human CD8<sup>+</sup> T cells, as CD137 is not expressed on resting CD8<sup>+</sup> T cells and its expression is reliably induced after 24 hours of stimulation.<sup>1,2</sup>

### 1.2 Applications

- Identification and enumeration of activated antigen-specific CD137<sup>+</sup> T cells by flow cytometry or fluorescence microscopy.
- Identification and enumeration of activated antigen-specific CD137<sup>+</sup> T cells in combination with a MACS® Cytokine Secretion Assay Kit.
- Evaluation of intracellular cytokine expression in activated antigen-specific CD137<sup>+</sup> T cells by using CD137 antibodies in combination with antibodies against human cytokines.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD137 conjugate	PE	APC	Biotin
Flow cytometry <sup>a</sup>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11
- Formaldehyde-fixed and permeabilized cells	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100 µL of buffer.

### 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 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.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), Anti-Biotin-APC (# 130-090-856), or Anti-Biotin-VioBlue® (# 130-094-669) as secondary antibody reagent in combination with CD137-Biotin.
- (Optional) CD8-PE (# 130-091-084) or CD8-APC (# 130-091-076). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (Optional) Stimulation reagents: CytoStim (# 130-092-172, # 130-092-173) as control reagent for T cell stimulation and PepTivator® – CMV pp65 (# 130-093-435, # 130-093-438) or the CMV pp65 – Recombinant Protein (# 130-091-823, # 130-091-824) for antigen-specific T cell stimulation.

- (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

### 2.1 Protocol for *in vitro* stimulation for induction of CD137 expression

▲ Always include a negative control in the experiment. The sample should be treated exactly the same way as the stimulated sample, except for the addition of the stimulus.

▲ A positive control may also be included in the experiment, such as a sample stimulated with CytoStim (# 130-092-172, # 130-092-173).

▲ Do not use media containing any non-human proteins, such as BSA or FBS, because of non-specific stimulation.

1. Wash cells by adding cell culture medium, centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cells at a density of 10<sup>7</sup> cells/mL in culture medium containing 5% human serum. Plate cells in dishes at a density of 5×10<sup>6</sup> cells/cm<sup>2</sup> (refer to Appendix).
3. Add an antigen or control reagent in the appropriate concentration, for example, PepTivator – CMV pp65 (# 130-093-435, # 130-093-438).
4. Incubate cells overnight with antigen and an appropriate control, e.g., CytoStim, at 37 °C and 5% CO<sub>2</sub>.
  - ▲ **Note:** Stimulation for 4–6 hours with CytoStim is sufficient to induce CD137 expression. For antigen-specific stimulation an overnight incubation is recommended.
5. Collect cells carefully by pipetting up and down when working with smaller volumes. Rinse the dish with cold buffer. Check microscopically for any remaining cells. If necessary, rinse the dish again.

### 2.2 Immunofluorescent staining

▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

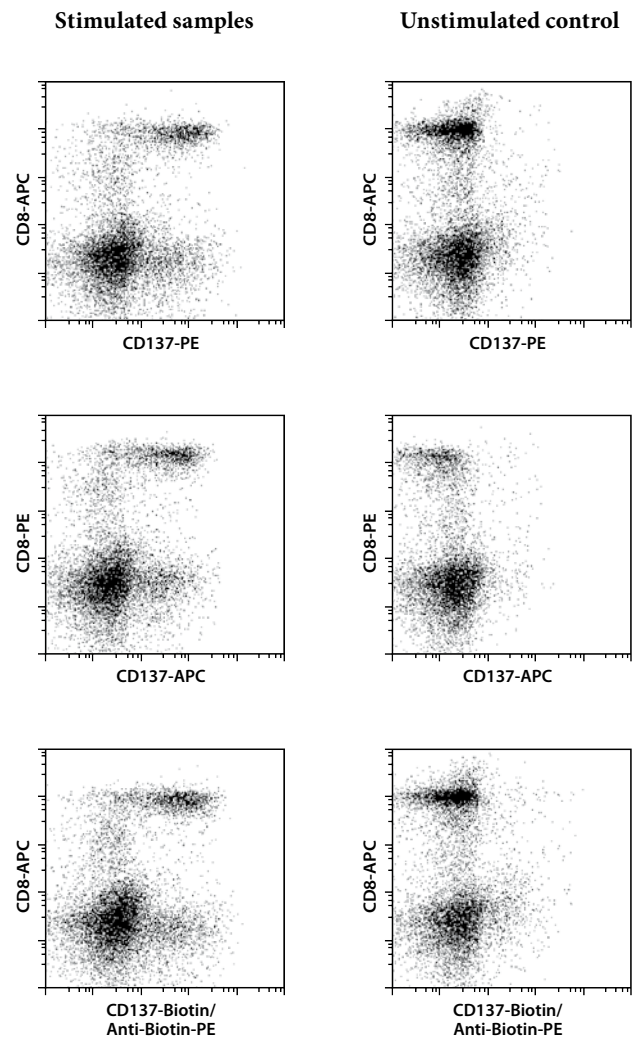
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD137 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **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 per 10<sup>7</sup> cells 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 (Anti-Biotin-FITC, Anti-Biotin-PE, Anti-Biotin-APC, or Anti-Biotin-VioBlue), 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

Human peripheral blood mononuclear cells (PBMCs) were incubated overnight with and without CytoStim. Subsequently, cells were stained with CD137 antibodies conjugated to PE or APC as well as with CD8-PE (# 130-091-084) or CD8-APC (# 130-091-076) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD137-Biotin were stained with Anti-Biotin-PE (# 130-090-756) as well as CD8-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. References

1. Wehler, T. C. *et al.* (2007) Targeting the activation-induced antigen CD137 can selectively deplete alloreactive T cells from antileukemic and antitumor donor T-cell lines. *Blood* 109(1): 365–373.
2. Wolf, M. *et al.* (2007) Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of CD8<sup>+</sup> T cells responding to antigen without requiring knowledge of epitope specificities. *Blood* 110(1): 201–210.

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

## 5. Appendix: Flask and dish sizes for *in vitro* stimulation of PBMCs

For *in vitro* stimulation of PBMCs (refer to 2.2) the cells should be resuspended in culture medium, containing 5% of human serum, at a dilution of  $10^7$  cells/mL. The cells should be plated at a density of  $5 \times 10^6$  cells/cm<sup>2</sup>. Both the dilution and the cell density are important to assure optimum stimulation.

The following table lists culture plate, dish and flask sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to add.

Total cell number	Medium volume to add	Culture plate	Well diameter
$0.15 \times 10^7$	0.15 mL	96 well	0.64 cm
$0.50 \times 10^7$	0.50 mL	48 well	1.13 cm
$1.00 \times 10^7$	1.00 mL	24 well	1.60 cm
$2.00 \times 10^7$	2.00 mL	12 well	2.26 cm
$5.00 \times 10^7$	5.00 mL	6 well	3.50 cm
Total cell number	Medium volume to add	Culture dish	Dish diameter
$4.5 \times 10^7$	4.5 mL	small	3.5 cm
$10.0 \times 10^7$	10.0 mL	medium	6 cm
$25.0 \times 10^7$	25.0 mL	large	10 cm
$50.0 \times 10^7$	50.0 mL	extra large	15 cm
Total cell number	Medium volume to add	Culture flask	Growth area
$12 \times 10^7$	12 mL	50 mL	25 cm <sup>2</sup>
$40 \times 10^7$	40 mL	250 mL	75 cm <sup>2</sup>
$80 \times 10^7$	80 mL	720 mL	162 cm <sup>2</sup>
$120 \times 10^7$	120 mL	900 mL	225 cm <sup>2</sup>

## 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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