Anti-THEMIS antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
Anti-THEMIS-PE	for 30 tests	130-108-271
Anti-THEMIS-PE	for 100 tests	130-108-244
Anti-THEMIS-APC	for 30 tests	130-108-272
Anti-THEMIS-APC	for 100 tests	130-108-245

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.

Technical data and background information

Antigen	THEMIS
Clone	REA463
Isotype	recombinant human IgG1
Isotype control	REA Control (I) antibodies
Alternative names of antigen	C6orf190, C6orf207, GASP, SPOT, TSEPA, THMS1, GRB2-associated protein
Molecular mass of antigen [kDa]	74
Distribution of antigen	T cells, thymocytes, T helper cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA463 recognizes the human thymocyte-expressed molecule involved in selection (THEMIS) antigen, a 73 kDa signaling protein, that is also known as thymocyte selection pathway associated (TSEPA), signaling phosphoprotein specific for T cells (SPOT), or GRB2-associated protein (GASP). THEMIS is rapidly tyrosine-phosphorylated upon TCR ligation and is required for the differentiation of immature CD4/CD8 double positive thymocytes into mature CD4 or CD8 single positive thymocytes. It is expressed only in the T cell lineage and is first detected in CD4/CD8 double negative thymocytes, reaching maximum levels at the CD4/CD8 double positive stage. Following thymocyte selection, THEMIS expression is decreased in mature CD4 and CD8 single positive thymocytes and peripheral T cells. Themis$^{-/-}$ mice show impaired positive selection during thymocyte development and severe reduction of mature thymocytes and peripheral T cells.
Additional information: Clone REA463 displays negligible binding to Fc receptors.

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 Ca^{2+} or Mg^{2+} are not recommended for use.
- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells containing Inside Fix and Inside Perm.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for intracellular staining of cells

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^7 cells/100 µL of buffer.
- It is recommended to stain 10^6 cells per sample. When working with up to 10^7 cells, use 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).
- The special protocol “Intracellular staining in combination with magnetic cell separation” is available for download at www.miltenyibiotec.com/protocols. In-column intracellular staining of cells immobilized on an MS Column is especially advantageous for the analysis of rare cells.

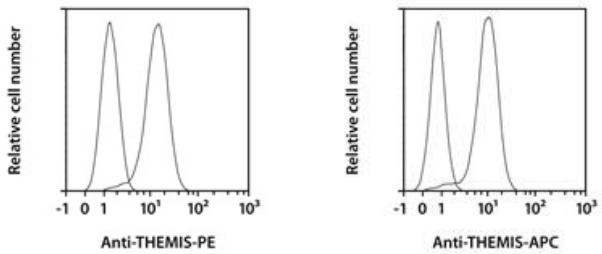
1. Wash up to 10^7 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 cells in 500 µL of buffer.
4. Add 500 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes in the dark at room temperature.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
   Note: Fixed cells may be stored in azide-containing buffer at 2–8 °C for up to 1 week.
7. (Optional) Stain cell surface antigens that are sensitive to permeabilization with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cells in 90 µL of Inside Perm. Add 10 µL of the antibody.
   Note: For staining with several antibodies in this step, reduce the volume of Inside Perm accordingly. For efficient permeabilization, the volume of Inside Perm should be at least 30% of the overall staining volume.
10. Mix well and incubate for 10 minutes in the dark at room temperature.
11. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
12. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of Inside Perm, add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 10 and 11.
13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

- Note: Samples may be stored at 2–8 °C in the dark for up to 24 hours.

- Note: Do not use propidium iodide (PI) or 7-AAD staining.

Examples of immunofluorescent staining

Human Jurkat cells were fixed, permeabilized, and stained with Anti-THEMIS antibodies or with the corresponding REA Control (I) antibodies (left peak). Flow cytometry was performed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals.



References

1. **Paster, W. *et al.*** (2013) GRB2-mediated recruitment of THEMIS to LAT is essential for thymocyte development. J. Immunol. 190(7): 3749–3756.
2. **Gascoigne, N. R. *et al.*** (2015) THEMIS: a critical TCR signal regulator for ligand discrimination. Curr. Opin. Immunol. 33: 86–92.
3. **Paster, W. *et al.*** (2015) A THEMIS: SHP1 complex promotes T-cell survival. EMBO J. 34(3): 393–409.

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