Miltenyi Biotec

Anti-Slug antibodies
human

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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 Monoclonal Anti-Slug antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
PE	130-106-135	130-106-185
APC	130-106-136	130-106-186

Clone REA404 (isotype control: REA Control (I)).

Capacity 1 mL: 100 tests or up to 10^8 total cells

300 µL: 30 tests or up to 3×10^7 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: Slug
- Synonym: SNAI2; SLUGH1; SNAIL2; WS2D; SLUG; SLUGH
- Expression patterns: Clone REA404 recognizes the human slug antigen, a zinc finger transcriptional repressor also known as SNAI2. Slug is an inducer of the epithelial to mesenchymal transition (EMT) which is a highly conserved developmental program activated during mesoderm formation and neural crest development. This program also promotes dissemination of single malignant cells from primary epithelial tumors. During EMT, cells discard their epithelial characteristics, including cell adhesion and polarity, reorganize their cytoskeleton and acquire a mesenchymal morphology and the ability to migrate. One of the hallmarks of EMT is the functional loss of the cell-cell junction protein E-cadherin. Several transcription factors including slug have been identified to repress E-cadherin expression, which plays important roles in cell adhesion and in the maintenance of tissue structure. These EMT transcription factors bind to E-box elements in the promoter region of E-cadherin leading to transcriptional repression of junctional complexes and induction of the mesenchymal phenotype. Additional information: Clone REA404 displays negligible binding to Fc receptors.

1.2 Applications

- Identification and enumeration of Slug$^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Slug conjugates is **1:11 for up to 10^6 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- FoxP3 Staining Buffer Set (# 130-093-142).

  ▲ **Note:** Use of the FoxP3 Staining Buffer Set is critical for optimal results. Always prepare solutions freshly and according to the data sheet supplied with the kit.

  ▲ **Caution:** Items within the FoxP3 Staining Buffer Set contain formaldehyde (EU Hazard Classification: Xn harmful; R40/20/21/22-43).

2. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

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▲ For optimal intracellular staining the FoxP3 Staining Buffer Set (# 130-093-142) must be used. Always prepare reagents freshly as recommended in the data sheet.

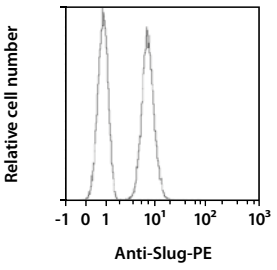
▲ Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells in 1 mL of cold, freshly prepared Fixation/Permeabilization Solution.
4. Mix well and incubate for 30 minutes in the dark in the refrigerator (2–8 °C).
5. Wash cells by adding 1–2 mL of cold buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
6. Wash cells by adding 1–2 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
7. Resuspend up to 10^6 nucleated cells in 100 µL of cold 1× Permeabilization Buffer.
8. Add 10 µL of the Anti-Slug antibody.
9. Mix well and incubate for 30 minutes in the dark in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
11. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

    ▲ **Note:** Due to fixation and permeabilization, cells are smaller than viable cells. Thus, FSC/SSC settings of the flow cytometer might need to be adjusted.

3. Examples of immunofluorescent staining with Anti-Slug antibodies

HeLa cells were fixed and permeabilized using the FoxP3 Staining Buffer Set. Cells were then stained with Anti-Slug antibodies or with the corresponding REA Control (I) antibodies (left peak) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris were excluded from the analysis based on scatter signals.



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

4. References

1. Guo, W. *et al.* (2012) Slug and Sox9 cooperatively determine the mammary stem cell state. Cell 148 (5): 1015–1028.
2. Hemavathy, K. *et al.* (2000) Human Slug is a repressor that localizes to sites of active transcription. Mol. Cell. Biol. 20 (14): 5087–5095.
3. Sánchez-Martín, M. *et al.* (2002) SLUG (SNAI2) deletions in patients with Waardenburg disease. Hum. Mol. Genet. 11 (25): 3231–3236.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty