Miltenyi Biotec

Anti-SSEA-4 antibodies
human

Contents

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-SSEA-4 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-098-371	–
PE	130-098-369	130-100-635
APC	130-098-347	–
VioBlue®	130-098-366	–
VioGreen™	130-098-341	–
PE-Vio770™	130-105-051	130-105-081
APC-Vio770™	130-105-052	130-105-082
PerCP-Vio700™	130-105-053	130-105-083
Biotin	130-098-339	–

Clone REA101 (isotype control: REA Control (S)).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 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.

Cross-reactivity: The Anti-SSEA-4 antibody has been reported to react with

- mouse cells
- rabbit cells
- canine cells
- chicken cells

1.1 Background information

- Antigen: SSEA-4
- Expression patterns: The monoclonal antibody REA101 recognizes the glycolipid carbohydrate epitope stage specific embryonic antigen 4 (SSEA-4). SSEA-4 is found on undifferentiated human embryonic stem (ES) cells, induced pluripotent (iPS) cells, embryonal carcinoma (EC) cells, and embryonic germ (EG) cells and a variety of somatic stem cells, such as dental pulp stem cells, umbilical cord blood-derived very small embryonic like stem cells (VSELs) and mesenchymal stromal cells. Additional information: Clone REA101 displays negligible binding to Fc receptors.

1.2 Applications

- Identification and enumeration of SSEA-4$^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-SSEA-4 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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.

- (Optional) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye–conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Anti-SSEA-4-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow

140-003-740.05

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

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

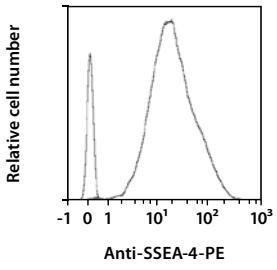
Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-SSEA-4 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 Anti-SSEA-4-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. Example of immunofluorescent staining with Anti-SSEA-4 antibodies

Human induced pluripotent stem (iPS) cells either unstained (left peak) or stained with Anti-SSEA-4 antibodies conjugated to PE were analyzed by flow cytometry using the MACSQuant® Analyzer. 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/antibodies.

4. References

1. Gang, E. J. *et al.* (2007) SSEA-4 identifies mesenchymal stem cells from bone marrow. Blood 109 (4): 1743–1751.
2. Truong, T. T. *et al.* (2011) SSEA4 is a potential negative marker for the enrichment of human corneal epithelial stem/progenitor cells. Invest. Ophthalmol. Vis. Sci. 52 (9): 6315–6320.
3. Kannagi, R. *et al.* (1983) Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside EMBO J. 2 (12): 2355–2361.

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

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

140-003-740.05