Miltenyi Biotec

PSC Analysis Cocktail Kit, anti-human

Order no. 130-136-292

Contents

1. Description

This product is for research use only.

Components

0.5 mL PSC Analysis Cocktail, anti-human containing:

CD15 Antibody, anti-human, VioBlue® (clone: VIMC6, isotype: mouse IgM)

SSEA-4 Antibody, anti-human, VioGreen™ (clone: REA101, isotype: recombinant human IgG1)

TRA-1-60 Antibody, anti-human, PE (clone: REA157, isotype: recombinant human IgG1)

Oct3/4 Antibody, anti-human/mouse, APC (clone: REA622, isotype: recombinant human IgG1)

0.5 mL PSC Control Cocktail, anti-human containing:

Isotype Control Antibody, mouse IgMκ, VioBlue (clone: IS5-20C4)

REA Control Antibody, human IgG1, VioGreen, REAfinity™ (clone: REA293)

REA Control Antibody, human IgG1, PE, REAfinity (clone: REA293)

REA Control Antibody, human IgG1, APC, REAfinity (clone: REA293)

FoxP3 Staining Buffer Set containing:

25 mL Fixation/Permeabilization Solution 1

2×40 mL Fixation/Permeabilization Solution 2

40 mL Permeabilization Buffer (10×)

0.3 mL SSEA-4 Antibody, anti-human, VioBlue (clone: REA101, isotype: recombinant human IgG1)

0.3 mL SSEA-4 Antibody, anti-human, VioGreen (clone: REA101, isotype: recombinant human IgG1)

0.3 mL TRA-1-60 Antibody, anti-human, PE (clone: REA157, isotype: recombinant human IgG1)

0.3 mL Oct3/4 Antibody, anti-human/mouse, APC (clone: REA622, isotype: recombinant human IgG1)

Capacity 50 tests or each 5×10^7 cells.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store 10× Permeabilization Buffer at room temperature. Store all other components protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

During the culture of pluripotent stem cells (PSCs), it is essential to constantly monitor the pluripotency status using multiple pluripotency-associated markers such as SSEA-4, TRA-1-60, and Oct3/4. The PSC Analysis Cocktail Kit, anti-human has been designed for the reliable enumeration and analysis of human PSCs using multicolor flow cytometry. The kit includes extracellular and intracellular markers essential for the reliable identification of PSCs and provides all buffer solutions and control reagents required for the protocol, e.g., single reagents for compensation. Furthermore, the integration of CD15 (SSEA-1) enables the detection of unwanted spontaneous differentiation of the culture. The panel design is compatible with the use of GFP-labeled stem cell lines or allows for additional integration of markers like NANOG or SOX2 using the blue laser (488 nm). Dead cell exclusion is enabled by the optional use of Viobility™ 640/770 Fixable Dye.

1.2 Applications

- Identification and phenotyping of cultured human PSCs based on recommended standards by the International Society for Stem Cell Research (ISSCR) regarding marker expression analyzed by flow cytometry

1.3 Reagent and instrument 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 to +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^{2+} or Mg^{2+} are not recommended for use.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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- Flow cytometer with a red (640 nm), a blue (488 nm) and a violet (405 nm) laser, e.g., MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) Viobility™ 640/770 Fixable Dye (# 130-134-046) for detection of dead cells during flow cytometric analysis.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Nanog Antibody, anti-human, Vio® B515, REAfinity™ and Sox2 Antibody, anti-human/mouse, FITC, REAfinity. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

2. Protocol

2.1 Reagent preparation

▲ Always prepare reagents freshly. Failure to do so may lead to suboptimal results.

▲ Total buffer volumes should be calculated beforehand and will depend on the number of samples.

Fixation/Permeabilization solution

To achieve the appropriate working concentration for safe fixation and permeabilization of cells, dilute Fixation/Permeabilization Solution 1 1:4 with the Fixation/Permeabilization Solution 2, i.e. for 10^6 cells use 0.25 mL of Fixation/Permeabilization Solution 1 and 0.75 mL of Fixation/Permeabilization Solution 2.

Permeabilization Buffer

To achieve the appropriate working concentration for safe permeabilization of cells, dilute Permeabilization Buffer (10×) 1:10 with deionized or distilled water before use, i.e. 1 mL of Permeabilization Buffer (10×) and 9 mL of deionized/distilled water.

2.2 Preparation of compensation controls

1. Determine cell number.
2. Prepare five tubes (VioGreen, VioBlue, PE, APC, blank), each with up to 5×10^5 cells.
3. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
4. Resuspend cells in 500 µL freshly prepared Fixation/Permeabilization solution, mix well and incubate for 15 minutes in the dark in the refrigerator (+2 to +8 °C).
5. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. Wash cells by adding 1 mL of Permeabilization Buffer (1×) and centrifuge at 300×g for 5 minutes at +4 °C. Aspirate supernatant completely.
7. Resuspend cells for blank in 100 µL of Permeabilization Buffer (1×).
8. Resuspend cells for VioGreen, VioBlue, PE, and APC compensation controls in 90 µL of Permeabilization Buffer (1×).
8. Add 10 µL of SSEA-4 Antibody, anti-human, VioGreen into the tube for VioGreen, 10 µL of SSEA-4 Antibody, anti-human, VioBlue into the tube for VioBlue, 10 µL of TRA-1-60 Antibody, anti-human, PE into the tube for PE and 10 µL of Oct3/4 Antibody, anti-human, APC into the tube for APC compensation controls.
9. Mix well and incubate for 30 minutes in the dark in the refrigerator (+2 to +8 °C).
10. Wash by adding 1 mL of Permeabilization Buffer (1×) and centrifuge at 300×g for 5 minutes.
11. Aspirate supernatant completely and resuspend each pellet separately in 500 µL of buffer. All tubes are ready-to-use.
12. Compensate instrument by following the instructions in the instrument user manual.

2.3 Immunofluorescent staining of cultured PSCs

▲ The following protocol is for working with cultured human PSCs. A protocol covering all steps from sample preparation and cell isolation to cell culture and flow cytometry is available at www.miltenyibiotec.com/applications/pluripotent-stem-cells.html.

▲ Cultured PSCs have to be dissociated with an appropriate dissociation reagent and used immediately.

▲ Volumes given below are for up to 5×10^5 cells. When working with fewer than 5×10^5 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 1×10^6 cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Prepare two tubes (tube A, tube B) with up to 5×10^5 cells each.
3. Centrifuge cell suspension at 300×g for 5 minutes.
4. Aspirate supernatant completely and resuspend each aliquot in 500 µL of freshly prepared Fixation/Permeabilization solution.
5. Mix well and incubate for 15 minutes in the dark in the refrigerator (+2 to +8 °C).
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
7. Wash cells by adding 1 mL of Permeabilization Buffer (1×) and centrifuge at 300×g for 5 minutes at +4 °C. Aspirate supernatant completely.
8. Resuspend the cells in 90 µL of Permeabilization Buffer (1×). Add 10 µL of PSC Analysis Cocktail, anti-human into tube A and 10 µL of PSC Control Cocktail, anti-human into tube B.
9. Mix well and incubate for 30 minutes in the dark in the refrigerator (+2 to +8 °C).

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

10. Wash cells by adding 1 mL of Permeabilization Buffer (1×) and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
11. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry. A volume of 500 µL of buffer per sample is recommended.

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2.4 Flow cytometric data acquisition

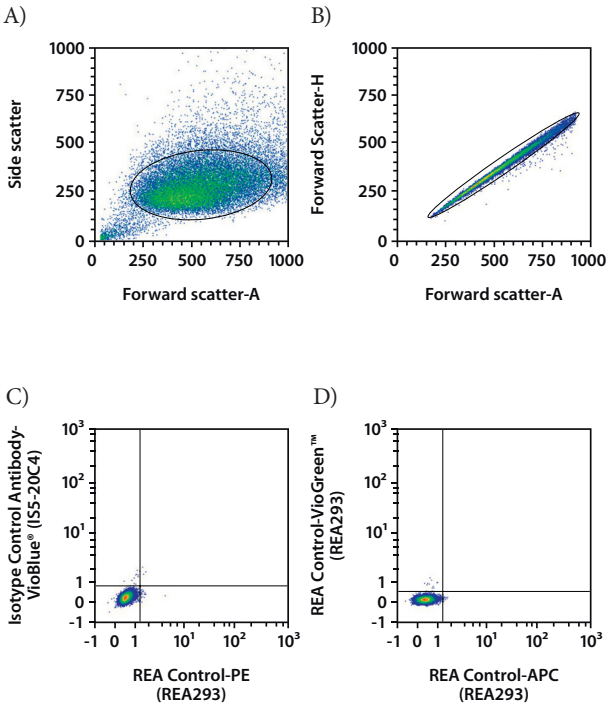
▲ Refer to the MACSQuant Instrument user manual and software guide for detailed information on using the MACSQuant Analyzer.

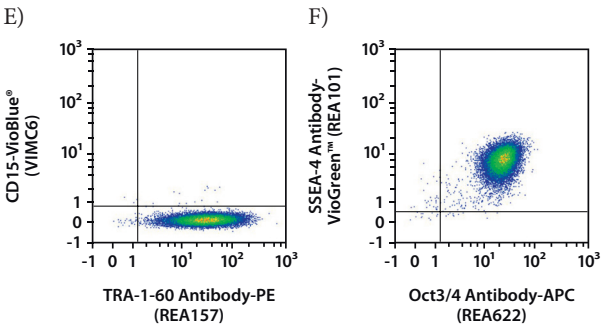
▲ The flow cytometer should be compensated for PSCs (refer to chapter 2.2).

1. Prepare and prime the MACSQuant Analyzer. Make sure the calibration and instrument settings of the instrument have been optimized for acquisition of the PSC Analysis Cocktail Kit, anti-human.
2. Analyze tube A (PSC Analysis Cocktail, anti-human) and tube B (PSC Control Cocktail, anti-human).
3. Define an appropriate threshold based on forward scatter (FSC) versus side scatter (SSC) to exclude debris from the data acquisition.
4. For doublet discrimination choose **Height**. Therefore click the **Advanced** button located in the **Channels** tab and click on the **Height** button.
5. Start flow cytometric data acquisition.

3. Example of immunofluorescent staining with the PSC Analysis Cocktail Kit, anti-human

Cultured PSCs were stained with the PSC Analysis Cocktail Kit, anti-human and analyzed by flow cytometry using the MACSQuant Analyzer 16. As a preliminary step for elimination of doublets a gate around single cells in forward scatter area (FSC-A) versus forward scatter height (FSC-H) was set. Cells stained with PSC Control Cocktail, anti-human were analyzed for the expression of the corresponding isotype controls which enables to set the gate for identifying positively stained cells (A–D). The cells stained with PSC Analysis Cocktail, anti-human were analyzed for expression of TRA-1-60, SSEA-4, Oct3/4, and CD15 (E–F.)





Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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