[Protocol 1.04.0] Sorting of differentiated cells using MACS

Author: Jeong-Eun Lee
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Protocol 1.04.0 Sorting of differentiated cells using MACS
Version: 1.0 (20.12.2021)

Media and Reagents:
Anti-SSEA4 MicroBeads, human (miltenyibiotec, 130-097-855)
Anti-TRA-1-60 MicroBeads, human (miltenyibiotec, 130-100-832)
optimised Medium for species (*refer to protocol 1.07, 1.08 or 1.09*)
TrypLE-Select (1x) (Thermo Fisher, 12563011) **or**
Accutase (Stem Cell Technologies, 07920 or Thermo Fisher A1110501)
for single cell split , refer to protocol 1.03.0
PBS w/o Ca2+Mg2+ (Thermo Fisher, 14190250)
Y-27632 ROCK inhibitor (Stem Cell Technologies,72305 or Selleck Chemicals, SEL-S1049-10mM)
add ROCK inhibitor regarding protocol 1.03.1
MACS Buffer (*refer to protocol 1.04.1*) Dulbecco's phosphate-buffered saline (DPBS) without Ca2+ and Mg2+, with 0.5% bovine serum albumin (BSA), and 2 mM EDTA. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column

Materials and Equipment:
MidiMACS™ Separator (miltenyibiotec, 130-042-302) Anti-SSEA4
MiniMACS™ Separator (miltenyibiotec, 130-042-102) Anti-TRA-1-60
MACS® MultiStand (miltenyibiotec,130-042-303)
LS Columns (miltenyibiotec,130-042-401) Anti-SSEA4
MS Columns (miltenyibiotec,130-042-201) Anti-TRA-1-60
Pre-Separation Filters (30 µm) (miltenyibiotec,130-041-407)
Geltrex coated culture vessels (*refer to protocol 1.05*)
Aspiration Pump
5ml/10ml pipettes
Cell counting equipment
15/50 ml Falcon tube

1. Introduction and Purpose
First, the TRA-1-60+/SSEA4+ cells are magnetically labeled with Anti-TRA-1-60/Anti-SSEA4 MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled TRA-1-60+/SSEA4+ cells are retained within the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained TRA-1-60+/SSEA4+ cells can be eluted as the positively selected cell fraction.

2. Sample preparation
1. Remove culture medium and wash culture dish twice with DPBS.
Note: Buffers or media containing Ca2+ or Mg2+ are not recommended for use.
2. Trypsinize with 1 mL of TrypLE per a well of the 6 WP for 5 minutes at 37 °C
for single cell split , refer to protocol 1.03.0
3. Stop enzymatic reaction by adding 2 mL of culture medium supplemented with ROCK inhibitor.
4. Dissociate to single-cell suspension by pipetting up and down using a 5 mL serological pipette.
5. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer or culture medium before use

3. Magnetic labeling
Note: Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 5 minutes.
3. Aspirate supernatant completely.
4. Re-suspend cell pellet in 80 µL culture medium supplemented with ROCK inhibitor per 2×10^6 total cells (Anti-TRA-1-60)/ per 10^7 total cells (Anti-SSEA4).
Note: Human ESCs and iPSCs should be magnetically labeled and separated in standard culture medium supplemented with ROCK inhibitor to achieve highest viability. However, for preparative separations the use of buffer is advisable.
Note: Volumes for magnetic labeling given below are for up to 2×10^6 total cells (Anti-TRA-1-60)/ 10^7 total cells (Anti-SSEA4). When working with fewer than 2×10^6 cells (Anti-TRA-1-60)/ 10^7 total cells (Anti-SSEA4), use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 4×10^6 total cells (Anti-TRA-1-60), use twice the volume of all indicated reagent volumes and total volumes)
5. Add 20 µL of Anti-TRA-1-60 MicroBeads per 2×10^6 total cells/ Add 20 µL of Anti-SSEA-4 MicroBeads per 107 total cells.
6. Mix well and incubate for 5 minutes (Anti-TRA-1-60)/ 15 minutes (Anti-SSEA4) in the refrigerator (2–8 °C).
Note: Higher temperatures and/or longer incubation times may lead to non- specific cell labeling. Working on ice is not recommended
7. (Anti-TRA-1-60): Adjust volume to 1mL using buffer or culture medium supplemented with ROCK inhibitor. *Proceed to magnetic separation*
7. (Anti-SSEA4): Wash cells by adding 1–2 mL of buffer per 10^7cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.

8. (Anti-SSEA4): Resuspend up to 10^8 cells in 500 µL of buffer. *Proceed to magnetic separation*
Note: For higher cell numbers, scale up buffer volume accordingly.

4. Magnetic separation
1. Place column in the magnetic field of a suitable MACS Separator. (Anti-TRA-1-60): MS Column, (Anti-SSEA4): LS Column
2. (Anti-TRA-1-60): Prepare MS Column by rinsing with 0.5 mL standard culture medium supplemented with ROCK inhibitor
2. (Anti-SSEA4): Prepare LS column by rinsing 3 mL culture medium supplemented with ROCK inhibitor
Note: If human ESCs and iPSCs are further cultivated after the separation, the magnetic labeling and the separation should be performed in standard culture medium supplemented with ROCK inhibitor.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×0.5 mL (Anti-TRA-1-60)/ 2x3 mL (Anti-SSEA4) of MACS buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.
Note: Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL (Anti-TRA-1-60)/5 mL (Anti-SSEA4) of culture medium supplemented with ROCK inhibitor onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. Perform cell counting to examine how many cells are left.
8. Seed 1.0 – 2.0 x 10^4 cells per cm^2 (e.g. in a 6 well plate seed 1.0 – 2.0 x10^5 cells/well) in Geltrex coated culture vessels with pre-warmed media supplemented with with ROCK inhibitor

Relevant applicable documents:
Protocol 1.03.0 Passaging of iPSC into single cells using TrypLE/Accutase
Protocol 1.03.1 Reconstitution, aliquoting and use of Y26732 ROCK inhibitor
Protocol 1.05 Geltrex coating of culture vessels
Protocol 1.07 Preparation of E8 medium
Protocol 1.08 Preparation of mTeSR1 medium
Protocol 1.09 Preparation of stemMACS/UPPS medium