Miltenyi Biotec

Anti-TRA-1-60 MicroBeads human

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1. Description

This product is for research use only.

Components	2 mL Anti-TRA-1-60 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human TRA-1-60 antibodies.
Capacity	For 2×10^6 cells/test, up to 100 separations. For 2×10^8 total cells.
Product format	Anti-TRA-1-60 MicroBeads, human are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS Separation

First, the TRA-1-60$^+$ cells are magnetically labeled with Anti-TRA-1-60 MicroBeads, human. 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$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of TRA-1-60$^+$ cells. After removing the column from the magnetic field, the magnetically retained TRA-1-60$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The Anti-TRA-1-60 MicroBeads have been developed for the separation of human pluripotent stem cells based on the expression of the TRA-1-60 antigen. The TRA-1-60 monoclonal antibody reacts with a pluripotent stem cell–specific antigen expressed on undifferentiated human embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, embryonal carcinoma (EC) cells, and embryonic germ (EG) cells. The expression of TRA-1-60 on human ES cells is downregulated upon differentiation. The TRA-1-60 antibody recognizes a neuraminidase-resistant carbohydrate epitope expressed on podocalyxin, a member of the CD34-related family of sialomucins. Podocalyxin is a transmembrane glycoprotein, which has been implicated in the development of aggressiveness in a variety of cancers including breast cancer and prostate cancer.

1.3 Applications

- Positive selection of undifferentiated pluripotent stem cells, i.e. human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).
- Enrichment of human iPSCs after reprogramming.

1.4 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). Degas buffer before use, as air bubbles could block the column.

  ▲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.

- MACS Columns and MACS Separators: TRA-1-60$^+$ cells can be enriched by using MS Columns. Positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	5×10^6	MiniMACS, OctoMACS, SuperMACS II
autoMACS	2×10^8	2×10^7	autoMACS Pro Separator

  ▲ **Note:** Column adapters are required to insert certain columns into the SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- Trypsin solution: 0.05% trypsin, 2 mM EDTA
- Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps
- Culture medium supplemented with Rho-associated kinase (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.

- (Optional) For subculturing, supplementation of the culture medium with StemMACS™ Y27632 (# 130-103-922) or StemMACS Thiazovivin (# 130-104-461) is strongly recommended for the first 2 days of cultivation.

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., TRA-1-60 Antibody, anti-human. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

2. Protocol

2.1 Sample preparation

▲ Human ESCs or human iPSCs are sensitive to singling and some precautions have to be taken in order to make cells amenable to single-cell dissociation.

Different protocols have been described to obtain single-cell suspensions of human ESCs/iPSCs, for example, by using trypsin[1] or accutase[2]. If cells are further cultivated after the separation, the magnetic labeling and separation should be performend in standard culture medium supplemented with ROCK inhibitor.

The use of Rho kinase (ROCK) inhibitors as medium components during subculturing is beneficial in order to obtain highly viable hESCs/iPSCs after single-cell dissociation.[3] We strongly recommend using 10 µM StemMACS Y27632 or 2 µM StemMACS Thiazovivin.

1. Remove culture medium and wash culture dish twice with DPBS.
2. Trypsinize with 2 mL of trypsin solution per 10 cm culture dish for 5 minutes at 37 °C.

   ▲ **Note:** For human ESC or iPSC alternative enzymes have been succesfully employed.
3. Stop enzymatic reaction by adding 8 mL of culture medium containing FBS or trypsin inhibitor.
4. Dissociate to single-cell suspension by pipetting up and down using a 10 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.



2.2 Magnetic labeling

▲ The following protocol has been optimized for the positive selection of human pluripotent stem cells only

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. 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 before use.

▲ Volumes for magnetic labeling given below are for up to 2×10^6 total cells. When working with fewer than 2×10^6 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 4×10^6 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ The recommended incubation temperature is +2 to +8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer or culture medium supplemented with ROCK inhibitor per 2×10^6 total cells.

   ▲ **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.
4. Add 20 µL of Anti-TRA-1-60 MicroBeads, human per 2×10^6 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (+2 to +8 °C).
6. Adjust volume to 1 mL using buffer or culture medium supplemented with ROCK inhibitor.

   ▲ **Note:** For enrichment of iPSC after reprogramming an additional washing step is recommended after magnetic labeling and before magnetic separation.
   a) Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
   b) Resuspend cell pellet in 1 mL of buffer or culture medium supplemented with ROCK inhibitor.
7. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ The use of an MS Column is highly recommended. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare MS Column by rinsing with 500 µL of buffer or standard 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 1 mL of cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer or culture medium

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supplemented with ROCK inhibitor. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL 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.

▲ **Note:** For subculturing, supplementation of the culture medium with 10 µM StemMACS Y27632 or 2 µM StemMACS Thiazovivin is strongly recommended for the first 2 days of cultivation.

2.4 Magnetic labeling and separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

▲ Buffers used for operating the autoMACS Separators should have a temperature of ≥ +10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction, position C = labeled (positive) fraction.

2.4.1 Magnetic labeling and separation using the autoMACS Pro Separator

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample.
4. For a standard separation choose one of the following programs:

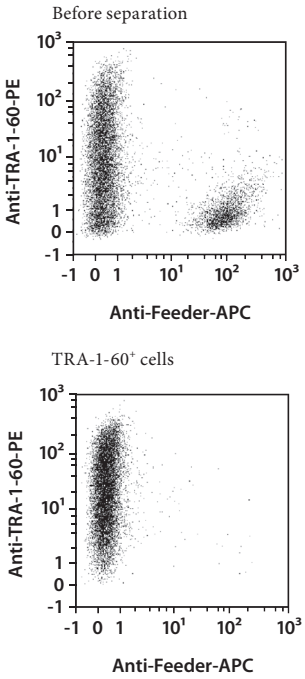
   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.
5. Tap **Run** to start the separation process.

Fully automated magnetic labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu. The correct labeling, separation, and wash protocols will be selected automatically.
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Tap **Run** to start the separation process.

3. Example of a separation using Anti-TRA-1-60 MicroBeads, human

Pluripotent (TRA-1-60$^+$) iPSCs were isolated from cultures containing spontaneously differentiated iPSCs grown on mouse embryonic feeder cells using Anti-TRA-1-60 MicroBeads, [human, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with Anti-TRA-1-60-PE and Anti-Feeder-APC after separation and 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.



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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