Miltenyi Biotec

Anti-TRA-1-60 MicroBeads
human

Order no. 130-100-832

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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	2 mL Anti-TRA-1-60 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human Anti-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 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 Principle of the MACS® Separation

First, the TRA-1-60$^+$ cells are magnetically labeled with Anti-TRA-1-60 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$^+$ 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$^+$ 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.[1] 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[2], 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: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}, 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.

  ▲ **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 total cells	Separator
Positive selection		
MS	5×10^6	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
autoMACS	2×10^7	autoMACS Pro

  ▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or 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

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- 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.

- (Optional) Fluorochrome-conjugated Anti-TRA-1-60 antibodies for flow cytometric analysis, e.g., Anti-TRA-1-60-PE (# 130-100-347). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

2. Protocol

2.1 Sample preparation

Harvesting of human ESCs or iPSCs cultured on feeder cells

▲ 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.

▲ 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.

▲ 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).

▲ 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.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice is not recommended.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 5 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 per 2×10^6 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 1 mL using 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.

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

Magnetic separation with MS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the MS Column data sheet.
2. Prepare MS Column by rinsing with 0.5 mL 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 3×0.5 mL of buffer or culture medium 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 only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

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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.

Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

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

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

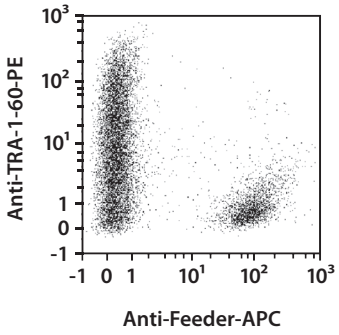
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For separation choose the following program:

   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

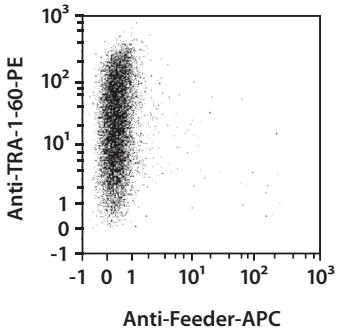
3. Example of a separation using Anti-TRA-1-60 MicroBeads

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, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with Anti-TRA-1-60-PE (# 130-100-347) and Anti-Feeder-APC (# 130-102-302) 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.

Before separation



Enriched TRA-1-60$^+$ pluripotent stem cells



4. References

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Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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