Miltenyi Biotec

StemMACS™ Trilineage Differentiation Kit

Order no. 130-115-660

Contents

1. Description

This product is for research use only.

Components	**36 mL StemMACS Trilineage MesoDiff Medium I** **72 mL StemMACS Trilineage MesoDiff Medium II** **60 mL StemMACS Trilineage EndoDiff Medium** **84 mL StemMACS Trilineage EctoDiff Medium**
Specifications	StemMACS Trilineage MesoDiff Medium I pH: 7.1–7.5 Osmolality: 270–310 mOsmol/kg StemMACS Trilineage MesoDiff Medium II pH: 7.1–7.5 Osmolality: 260–300 mOsmol/kg StemMACS Trilineage EndoDiff Medium pH: 7.1–7.5 Osmolality: 300–340 mOsmol/kg StemMACS Trilineage EctoDiff Medium pH: 7.1–7.5 Osmolality: 270–310 mOsmol/kg Functionality assay: Differentiation of human pluripotent stem cells into mesoderm, endoderm, and ectoderm.
Capacity	For 12 assays.
Storage	Upon arrival store the StemMACS Trilineage Media protected from light at –20 °C. The expiration date is indicated on the vial label. Aliquots of the media can be stored at –20 °C. Avoid repeated freeze-thaw-cycles. Once thawed, media bottles or aliquots should be kept at 2–8 °C and used within 2 weeks.

1.1 Background information

Pluripotency is the ability to differentiate into the three embryonal germ layers, ectoderm, mesoderm, and endoderm, and is a defining characteristic of pluripotent stem cells (PSCs). Therefore, basic characterization of PSC lines includes typically a test for pluripotency in addition to surface marker expression and morphology. However, traditional pluripotency assays such as embryoid body and teratoma formation are both time-consuming and difficult to quantitate.

The StemMACS Trilineage Differentiation Kit provides a functional assay that can be completed in 7 days. In contrast to other pluripotency assays, the StemMACS Trilineage Differentiation Kit allows either analysis of cells by immunocytochemistry or quantitative analysis by flow cytometry. The kit enables parallel assessment of multiple PSC lines. The 12-well format is optimal for flow cytometric analysis. For immunocytochemistry, the 24-well format may be sufficient.

1.2 Applications

- Assessment of differentiation potential of human pluripotent stem cells.

1.3 Reagent requirements

- Dulbecco's phosphate-buffered saline (D-PBS) with Ca^{2+} and Mg^{2+}
- Dulbecco's phosphate-buffered saline (D-PBS) without Ca^{2+} and Mg^{2+}
- 0.05% Trypsin/EDTA
- Soybean Trypsin Inhibitor (0.5 mg/mL)
- Corning® Matrigel® hESC-Qualified Matrix
- DMEM/F12 with L-Glutamin, without HEPES
- ROCK inhibitor, e. g. StemMACS Thiazovivin (# 130-104-461) or StemMACS Y27632 (# 130-103-922)
- PSC cultivation medium, e. g. StemMACS iPS-Brew XF (# 130-104-368)

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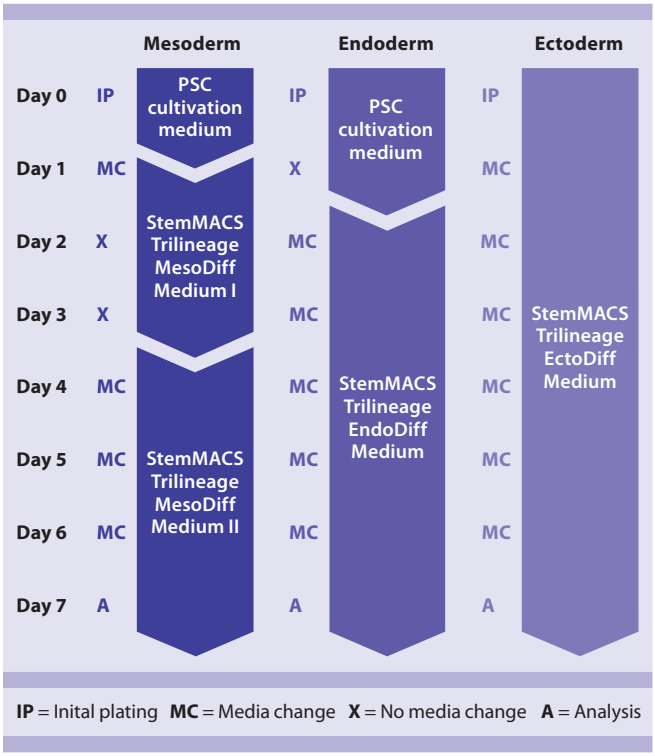
2. Protocols

2.1 Protocol overview



The assay starts with dissociation of the PSC culture into single-cell suspensions and seeding of defined cell numbers into 12- or 24-well plates in the presence of a ROCK inhibitor.

For mesoderm, cells are initially cultured for 1 day in PSC cultivation medium before mesoderm is induced using StemMACS Trilineage MesoDiff Medium I. Media changes are not required on day 2 and 3. From day 4 to day 6 media changes are performed daily using StemMACS Trilineage MesoDiff Medium II. On day 7, the differentiated samples are analyzed by either flow cytometry or immunocytochemistry.

For endoderm, cells are initially cultured in PSC cultivation medium for 2 days. Afterwards, endoderm differentiation is induced by exchanging the culture media with StemMACS Trilineage EndoDiff Medium followed by daily media changes.

For ectoderm, cells are differentiated in StemMACS Trilineage EctoDiff Medium with daily media changes.

2.2 Preparation of media

Thaw all media at 2–8 °C overnight.

2.3 Preparation of Matrigel®-coated plates

Coat plates according to the manufacturer's recommendation using a 1:80 dilution.

2.4 Harvesting of human pluripotent and differentiated cells

▲ It is mandatory to use single-cell suspensions for this assay. Volumes below are given for PSC maintenance cultivation in a 6-well plate. If using other culture ware adjust the volumes accordingly.

1. Aspirate cell culture medium and wash each well with 3 mL of D-PBS without Ca^{2+} and Mg^{2+}.
2. Add 1 mL of 0.05% Trypsin/EDTA per well. Gently rock the plate to ensure even distribution of the enzyme solution.
3. Incubate for 5 minutes at 37 °C in the dark.
4. Stop enzymatic reaction by adding 2 mL of Soybean Trypsin Inhibitor (0.5 mg/mL) per well.
5. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
6. Determine cell number.

2.5 Detailed differentiation protocol

Day 0

1. Plate cells by transferring the desired cell numbers into three 15 mL conical tubes. See table 1 for recommendations.
   ▲ **Note:** If using other culture ware use 40,000 cells/cm² for mesoderm, 66,000 cells/cm² for endoderm and 53,000 cells/cm² for ectoderm. Cell numbers might have to be adjusted depending on the cell line used.

Lineage	12-well plate	24-well plate
Mesoderm	150,000	80,000
Endoderm	250,000	130,000
Ectoderm	200,000	100,000

Table 1: Recommended starting cell numbers for mesoderm, endoderm, and ectoderm differentiation.

2. Centrifuge for 5 minutes at 200×g.
3. Aspirate supernatant.
4. Supplement media with ROCK inhibitor (10 µM StemMACS Y27632 or 2 µM StemMACS Thiazovivin) and resuspend the cell pellets using the media and volumes indicated in table 2.
   ▲ **Note:** Use Rock inhibitor only for initial plating.

Lineage	12-well plate	24-well plate	Medium
Mesoderm	1 mL	0.5 mL	PSC cultivation medium
Endoderm	1 mL	0.5 mL	PSC cultivation medium
Ectoderm	1 mL	0.5 mL	StemMACS Trilineage EctoDiff Medium

Table 2: Media and volumes used for plating.

5. Transfer the cells into the Matrigel-coated cell culture plate.

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Media changes

Mesoderm

Day 1

After 24 hours (day 1), replace the PSC cultivation medium with 3 mL StemMACS MesoDiff Medium I per well in a 12-well plate (or 1.5 mL per well in a 24-well plate).

Days 2–3

Do not change the differentiation medium on days 2 and 3.

Days 4–6

On days 4, 5, and 6 replace the medium daily with 2 mL StemMACS MesoDiff Medium II per well in a 12-well plate (or 1 mL per well in a 24-well plate).

Endoderm

Day 1

Do not change the PSC cultivation medium on day 1.

Days 2–6

Wash the cells daily using 2 mL D-PBS with Ca^{2+} and Mg^{2+} and add 1 mL StemMACS EndoDiff Medium per well in a 12-well plate (or 0.5 mL per well in a 24-well plate).

Ectoderm

Days 1–6

Change medium daily using 1 mL StemMACS Trilineage EctoDiff Medium per well in a 12-well plate (or 0.5 mL per well in a 24-well plate).

Analysis

On day 7, cells from all three differentiation pathways can be analyzed using either immunocytochemistry or flow cytometry.

For immunocytochemistry analysis fix cells, for example, with 4% paraformaldehyd (PFA) and stain according to manufacturer's recommendations. For marker recommendations see below.

For flow cytometry analysis harvest cells following the protocol in section 2.4 with half of the mentioned volumes by using a 1000 µL micropipette. A detailed protocol for flow cytometric staining and analyses is available at www.miltenyibiotec.com/130-115-660.

Marker recommendations

The following markers are suitable for analysis:

Mesodermal differentiation potential is described by the formation of endothelial cells ($CD144^+$) and/or the presence of smooth muscle cells ($CD140b^+$ or Smooth muscle 22 $alpha^+$). The ratio of endothelial and/or smooth muscle cells may vary between cell lines.

Endodermal differentiation capacity is characterized by the presence of CD184 $(CXCR4)^+Sox17^+$ definitive endoderm cells. A further marker that can be used is FoxA2.

Ectodermal differentiation potential can be assessed by the presence of $Pax6^+Sox2^+$ neuroectoderm cells.

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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