Miltenyi Biotec

StemMACS™ EndothelDiff Kit XF human

Order no. 130-139-194

Contents

1. Description

This product is for research use only.

Components	**500 mL StemMACS PSC-Brew Basal Medium XF, human** **4 mL StemMACS EndothelDiff Supplement I XF (50×), human** **7 mL StemMACS EndothelDiff Supplement II XF (50×), human** **125 mL StemMACS Endothel Cultivation Basal Medium XF, human** **5 mL StemMACS Endothel Cultivation Supplement XF (25×), human**
Specifications	Prepared Endothelial Diff Medium I Osmolality: 300–370 mOsmol/kg pH: 7.1–7.5 Prepared Endothelial Diff Medium II Osmolality: 300–370 mOsmol/kg pH: 7.1–7.5 Prepared Endothelial Cultivation Medium Osmolality: 260–330 mOsmol/kg pH: 7.1–7.5
Capacity	For 55 assays. One assay corresponds to one well of a 12-well plate.
Storage	Upon arrival, store StemMACS PSC-Brew Basal Medium XF, human and StemMACS Endothel Cultivation Basal Medium XF, human protected from light at +2 to +8 °C. Store StemMACS EndothelDiff Supplement I XF (50×), human, StemMACS EndothelDiff Supplement II XF (50×), human, and StemMACS Endothel Cultivation Supplement XF (25×), human protected from light at –20 °C. The expiration date is indicated on the label. Avoid repeated freeze-thaw-cycles. Once prepared, keep the complete media at +2 to +8 °C. UseEndothelDiff I and II within 2 weeks and Endothel Cultivation Medium within 3 weeks .

1.1 Background information

Directed differentiation of specific lineages from human pluripotent stem cells (hPSCs) is a major tool for developmental or disease models, drug screening platforms and cellular therapies. StemMACS EndothelDiff Kit XF, human is a complete, ready-to-use, and xeno-free cell culture system for the efficient and fast differentiation of hPSCs into endothelial cells. The kit is composed of two media that progressively restrict the cellular fate and promote the differentiation into endothelial cells in just 7 days of culture. hPSC-derived endothelial cells can be further expanded in StemMACS Endothel Cultivation Medium XF, human (# 130-139-193) and cultivated for more than 14 days.

1.2 Applications

- Directed differentiation of endothelial cells from hPSC lines.

1.3 Reagent and instrument requirements

- StemMACS iPS-Brew XF, human (# 130-104-368) or StemMACS PSC-Brew XF, human (# 130-127-865)
- ROCK inhibitor, e.g., StemMACS Thiazovivin (# 130-104-461), to improve cell attachment and survival.
- Dulbecco's phosphate-buffered saline (D-PBS) without Ca^{2+} and Mg^{2+}
- 0.05% Trypsin/EDTA or TrypLE™ and soybean trypsin inhibitor (0.5 mg/mL) for PSC endothelial cultivated cells
- Accutase® solution for PSC endothelial differentiated cells
- Cell attachment substrate for differentiation, e.g., MACSmatrix Laminin 511 (# 130-136-454) or Corning® Matrigel® hESC-qualified Matrix.
- Cell attachment substrate for cultivation, e.g., Laminin-411-E8 fragment or Collagen I
- (Optional) CD144 (VE-Cadherin) MicroBeads, human, (# 130-097-857)
- (Optional) iPSC-EC marker panel for flow cytometric analysis:

Specificity	Clone	Fluorochrome
CD105	REA794	Vio® Bright V423
CD34	REA1164	VioGreen™
CD144	REA199	FITC
CD309 (VEGFR-2)	REA1046	PE
CD140b	REA363	APC

Table 1: iPSC-EC marker panel.

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2. Protocol

2.1 Protocol overview

Day	Action	Complete medium
–1	Coat plates	
0	Plate iPSCs for endothelial differentiation	iPS-Brew or PSC-Brew complete medium with ROCK inhibitor*
1	Change media	EndothelDiff Medium I*
4	Change media	EndothelDiff Medium II*
5	Change media	
6	Change media, coat plates for iPS-endothelial cell cultivation	
7	Harvest cells and plate cells for iPS-endothelial cell cultivation	Endothel Cultivation Medium*

* For media preparation refer to table 3.

Table 2: Protocol overview.

2.2 Preparation of complete media

▲ Kit components should not be substituted or mixed with those from other kits or lots.

▲ Avoid repeated freeze-thaw cycles.

Complete media	Component	Amount (mL)
iPS-Brew or PSC-Brew Medium	StemMACS iPS-Brew or PSC-Brew Basal Medium XF, human	500
	StemMACS iPS or PSC-Brew Supplement XF, human	10
	Add rock inhibitor as indicated in the corresponding data sheet.	
EndothelDiff Medium I	StemMACS PSC-Brew Basal Medium XF, human	170
	StemMACS EndothelDiff Supplement I XF (50×), human	3.4
EndothelDiff Medium II	StemMACS PSC-Brew Basal Medium XF, human	330
	StemMACS EndothelDiff Supplement II XF (50×), human	6.6
Endothel Cultivation Medium	StemMACS Endothel Cultivation Basal Medium XF, human	125
	StemMACS Endothel Cultivation Supplement XF (25×), human	5

Table 3: Preparation of complete media.

1. Thaw supplements at +2 to +8 °C overnight.
   ▲ **Note:** If a white precipitate occurs, pipette up and down to dissolve it.
2. Shake the supplements vials vigorously.
3. Prepare complete media according to table 3.
   ▲ **Note:** (Optional) Antibiotic solutions might be added to prevent contamination, e.g. 100 U/mL penicillin and 100 µg/ mL streptomycin.
4. Complete media can be stored for up to 2 weeks (EndothelDiff Medium I and II) or up to 3 weeks (Endothel Cultivation Medium) at +2 to +8 °C.

2.3 Detailed differentiation protocol

▲ To achieve an efficient differentiation, it is important to start the differentiation with high quality PSCs. Regularly monitor the pluripotency status of the stem cell cultures by observing their morphology and staining for pluripotency associated markers. The PSC culture should show a confluency of 75–85% before starting (day 0).

Day –1

Coat 12-well plates with MACSmatrix Laminin 511 (0.35 µg/cm²) or Matrigel according to the manufacturer's recommendation

Day 0

Harvesting and plating of human pluripotent cells

When using a new stem cell clone it is strongly recommended to perform a titration experiment in order to determine the best starting PSC number for differentiation. Suggested cell number is 150,000 cells/12 well.

▲ Warm coated plates at room temperature.

▲ Prepare media as indicated in table 3.

▲ Volumes given below are for PSCs originally cultivated in a 6-well plate and transferred to a 12-well plate for induction. If using other culture ware adjust the volumes accordingly.

1. Aspirate cell culture medium and wash each well of the 6-well plate with 2 mL of D-PBS without Ca^{2+} and Mg^{2+} to harvest the cells.
2. Add 1 mL/well 0.05% Trypsin/EDTA per well (alternatively TrypLE). Gently rock the plate to ensure even distribution of the solution.
3. Incubate for 5 minutes in the dark at +37 °C.
4. Stop enzymatic reaction by adding 1 mL/well of soybean trypsin inhibitor (0.5 mg/mL).
5. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
6. Determine the cell number and viability. Viability should be >95%.
7. Transfer the desired cell number into a new tube. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
8. Resuspend the cells in a sufficient amount of iPS-Brew or PSC-Brew Medium supplemented with ROCK inhibitor (e.g., 2 µM StemMACS Thiazovivin). Per well, 1 mL of iPS-Brew or PSC-Brew Medium are needed.
9. Aspirate coating solution from new prepared 12-well plate.
10. Transfer cells into a pre-coated 12-well plate with 1 mL/well. Place the plate into the incubator (+37 °C, 5% CO_2).

Day 1

Replacing medium with EndothelDiff Medium I

1. One day (24 hours) after seeding, aspirate iPS-Brew or PSC-Brew Medium from the culture plate.
2. Replace with 3 mL/well of EndothelDiff Medium I.

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Day 4
Replacing medium with EndothelDiff Medium II

1. Three days after previous feeding, aspirate EndothelDiff Medium I from the culture plate.
2. Replace with 2 mL/well of EndothelDiff Medium II.

Day 5–6
Exchanging EndothelDiff Medium II daily

1. Replace old EndothelDiff Medium II with 2 mL/well fresh medium every 24 hours.

Day 7
Harvesting of cells

1. Aspirate cell culture medium and wash each well of the 12-well plate with 1 mL of D-PBS without Ca^{2+} and Mg^{2+}.
2. Add 0.5 mL/well Accutase solution per well. Gently rock the plate to ensure even distribution of the solution.
3. Incubate for 5 minutes in the dark at +37 °C.
4. Stop enzymatic reaction by adding 0.5 mL/well of D-PBS without Ca^{2+} and Mg^{2+}.
5. Centrifuge at 300×g for 5 minuts. Aspirate supernatant completely.
6. Resuspend cells in 1 mL/well of Endothel Cultivation Medium.
7. After cell counting, cells can be further cultivated (refer to section 2.4), analysed by flow cytometry, or used for other assays like lipid uptake assays or tube formation assays.

▲ **Note:** To improve purity of PSC derived endothelial cells , it is recommended to use CD144 (VE-Cadherin) MicroBeads, human as indicated in the data sheet. Especially if the frequency is below 50% of endothelial cells after differentiation, a subsequent cultivation of cells with high purity is improved when performing an magnetic enrichment step using CD144 (VE-Cadherin) MicroBeads, human.

2.4 Detailed cultivation protocol

▲ Warm coated plates at room temperature.

▲ Prepare Endothel Cultivation Medium as indicated in table 3.

Day –1

1. Coat 6-well plates with Laminin-411-E8 fragment or Collagen I according to the manufacturer's recommendation.

Day 0–4

1. Transfer harvested cells into a new tube and adjust cell concentration as needed with Endothel Cultivation Medium. The optimal seeding density is 10.000–20.000 cells/cm² to reach confluency within 7 days. Aspirate supernatant completely.
2. Resuspend cells in 2 mL of Endothel Cultivation Medium.
3. Place the plate into the incubator (+37 °C, 5% CO_2).
4. Perform a full media change after 3–4 days with 2 mL of Endothel Cultivation Medium.
5. Start harvesting when cells reach 100% confluency.

Day 7 (after reaching confluency)
Harvesting protocol

1. Aspirate cell culture medium and wash each well of a 6-well plate with 2 mL of D-PBS without Ca^{2+} and Mg^{2+}.
2. Add 1 mL/well TrypLE. Gently rock the plate to ensure even distribution of the solution.
3. Incubate for 5 minutes in the dark at +37 °C.
4. Stop enzymatic reaction by adding 0.5 mL/well of D-PBS without Ca^{2+} and Mg^{2+}
5. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
6. Determine the cell number and viability. Viability should be >95%.
7. Transfer the desired cell number into a new tube. Centrifuge at 300×g for 5 minuts. Aspirate supernatant completely.
8. Resuspend cells in 2 mL of Endothel Cultivation Medium.

After cell counting, cells can be further cultivated (refer to section 2.4), analysed by flow cytometry, or used for other assays like lipid uptake assays or tube formation assays.

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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