Miltenyi Biotec

StemMACS™ iPS-Brew XF human

Order no. 130-104-368

Contents

1. Description

Components	**500 mL StemMACS iPS-Brew XF, Basal Medium** **10 mL StemMACS iPS-Brew XF, 50× Supplement**
Specifications	pH: 7.2–7.6 Osmolality: 300–340 mOsmol/kg
Quality control	Maintenance of human iPS cell morphology and surface phenotype (TRA-1-60$^+$, SSEA-4$^+$) over five continuous passages. Low endotoxin level by Limulus Amoebocyte Lysate (LAL) assay. Tested negative for mycoplasma.
Storage	Store the StemMACS iPS-Brew XF, Basal Medium protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. Upon arrival store StemMACS iPS-Brew XF, 50× Supplement at –20 °C. Aliquots of the supplemented complete media can be stored at –20 °C for up to 2 month. Avoid repeated freeze-thaw-cycles. Once thawed, aliquots should be kept at 2–8 °C and be used within 2 weeks. The expiration date is indicated on the vial label.

Intended use
StemMACS iPS-Brew XF is intended for research use. It is not intended for human or animal diagnostic or therapeutic use.

1.1 Background information

StemMACS iPS-Brew XF is a xeno-free media formulation for the maintenance and expansion of human pluripotent stem cells under feeder-free conditions. StemMACS iPS-Brew XF supports rapid adaption of feeder-based cultures to a feeder-free environment. The formulation is compatible with standard cell attachment matrices, e.g. Matrigel® or vitronectin. It enables robust and efficient expansion of human embryonic stem cells (ES) or induced pluripotent stem cells (iPS) over multiple passages while maintaining a pluripotent phenotype as well as pluripotent differentiation potential. StemMACS iPS-Brew XF allows rapid culture re-initiation of pluripotent stem cell cultures after cryopreservation.

1.2 Applications

- Culture of human ES or iPS cells under xeno- and feeder-free conditions
- Rapid and easy adaption of feeder-based culture to a feeder-free environment
- Rapid culture initiation after cryopreservation

1.3 Reagent requirements

- Buffer: Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} and Mg^{2+}.
- A small molecule ROCK inhibitor, e.g., StemMACS Y27632 (# 130-103-922) or StemMACS Thiazovivin (# 130-104-461) to improve cell attachment and survival.
- StemMACS Passaging Solution XF (# 130-104-688) for passaging in cell clusters.
- 0.05% Trypsin/EDTA (alternatively, Accutase® or TrypLE™) and Soybean Trypsin Inhibitor (0.5 mg/mL) for single cell splitting.
- Cell attachment substrate. Validated substrates are, e.g., Matrigel®, Geltrex®, Laminin-511, Laminin-521, iMatrix-511, vitronectin, or CTS™ CELLstart™ substrate
- 15 mL conical tubes

2. Protocol

2.1 Preparation of complete media

Before StemMACS iPS-Brew XF can be used in cell culture, the two kit components need to be mixed according to the following protocol to obtain the complete medium.

1. Thaw StemMACS iPS-Brew XF, 50× Supplement at 2–8 °C prior to use.
2. To obtain the complete medium add 10 mL StemMACS iPS-Brew XF, 50× Supplement to 500 mL StemMACS iPS-Brew XF, Basal Medium. Mix well. The media is ready-to-use now. Use the complete medium within 2 weeks when stored at 2–8 °C.
3. For longer storage, prepare 50 mL aliquots and store at –20 °C for up to 2 month. Thaw aliquots of complete medium overnight at 2–8 °C. Once thawed, keep aliquots at 2–8 °C and use within 2 weeks.

2.2 Passaging of human ES or iPS cells

▲ StemMACS iPS-Brew XF is compatible with standard passaging techniques such as traditional colony cutting, passaging in cell clusters or single cells. It is recommended to use the single-cell or cluster-splitting technique in the presence of a small molecule ROCK inhibitor.

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Protocol for passaging in cell clusters

1. Coat 6-well plates with an appropriate attachment substrate according to the manufacturer's instructions.
2. Aspirate the cell culture supernatant.
3. Wash the cell layer with 3 mL of buffer per well.
4. Add 1 mL of StemMACS Passaging Solution XF per well. Gently rock the plate to distribute the solution evenly.
5. Incubate at room temperature for 4 minutes. Monitor the detachment process under the microscope.

   ▲ **Note:** Colonies must not detach completely. Only wait until the colony edges lift off (see figure 2).

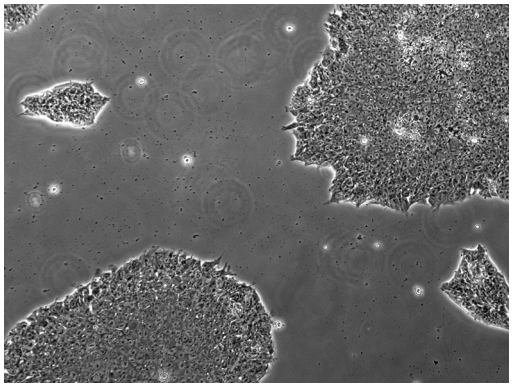

Figure 1: Colonies before addition of StemMACS Passaging Solution XF.

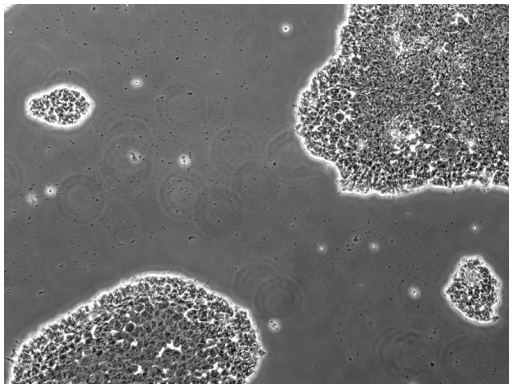

Figure 2: After 4 minutes incubation, colony edges start to lift off . At this point, the passaging solution should be removed.

6. Carefully remove the StemMACS Passaging Solution XF.
7. Per well, add 3 mL of StemMACS iPS-Brew XF supplemented with ROCK inhibitor (e.g., 2 µM StemMACS Thiazovivin or 10 µM StemMACS Y27632).
8. Gently detach the colonies by rinsing the well with a 5 mL serological pipette.
9. Transfer the cell suspension into a 15 mL conical tube.
10. Carefully pipette up and down 2–3 times to break up the colonies into smaller cell clusters.

    ▲ **Note:** Take care to minimize break-up of colonies. Do not create single cells!

11. Transfer the cell clusters into a fresh, appropriately coated 6-well cell culture plate. Use 2 mL StemMACS iPS-Brew XF supplemented with ROCK inhibitor per well and a splitting ratio between 1:6 and 1:20.

    ▲ **Note:** The optimal splitting ratio will depend on the cell line and must be determined empirically.

12. After 48 hours, replace media with fresh StemMACS iPS-Brew XF without ROCK inhibitor and continue with daily media changes.

    ▲ **Note:** Many ES and iPS cell lines will also tolerate every-other-day media changes when using StemMACS iPS-Brew XF.

Protocol for single-cell splitting

1. Coat 6-well plates with an appropriate attachment substrate according to the manufacturer's instructions.
2. Aspirate cell medium, wash each well with 3 mL of buffer.
3. Add 0.7 mL of 0.05% Trypsin/EDTA per well (alternatively, use Accutase or TrypLE). Gently rock the plate to ensure even distribution of the enzyme solution.
4. Incubate for 5 minutes at 37 °C.
5. Stop enzymatic reaction by adding 2 mL of Soybean Trypsin Inhibitor (0.5 mg/mL) per well.
6. Using a 5 mL serological pipette, dissociate to a single-cell suspension by carefully pipetting up and down.
7. Determine cell number.
8. Depending on the cell line, seed 70,000–150,000 cells per well (7000–16,000 cells/cm²). Transfer the desired cell number into a 15 mL conical tube.
9. Centrifuge for 5 minutes at 200×g.
10. Aspirate supernatant.
11. Resuspend the cell pellet in StemMACS iPS-Brew XF supplemented with a small molecule ROCK inhibitor (10 µM StemMACS Y27632 or 2 µM StemMACS Thiazovivin). Use 2 mL medium per well.
12. After 48 hours, replace media with fresh StemMACS iPS-Brew XF without ROCK inhibitor and continue with daily media changes.

    ▲ **Note:** Many ES and iPS cell lines will also tolerate every-other-day media changes when using StemMACS iPS-Brew XF.

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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