Miltenyi Biotec

# StemMACS™ Passaging Solution XF human

Order no. 130-104-688

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## 1. Description

**This product is for research use only.**

| | |
|---|---|
| **Components** | 100 mL StemMACS Passaging Solution XF, human |
| **Specifications** | Osmolality: 260–300 mOsmol/kg<br>pH: 7.2–7.6 |
| **Storage** | Store at room temperature. The expiration date is indicated on the vial label. |

### 1.1 Background information

StemMACS Passaging Solution XF, human is a xeno-free solution for routine passaging of human pluripotent stem cell cultures. The reagent enables the gentle detachment of human ES or iPS cell colonies and allows efficient dissociation into cell clusters while maintaining maximum viability. StemMACS Passaging Solution XF comes with a quick and simple protocol that minimizes manipulation of the culture. Lengthy inactivation, dilution or centrifugation steps are not required. Thus, cells can be quickly transferred into the new culture conditions ensuring optimal viability and attachment. The ready-to-use formulation facilitates a reproducible and standardized splitting procedure.
StemMACS Passaging Solution XF has been developed for use with StemMACS iPS-Brew XF, human, a xeno-free medium for feeder-free culture of human ES or iPS cells. The StemMACS XF Culture System supports long-term maintenance of a pluripotent phenotype, including typical undifferentiated cell morphology, pluripotent marker expression profile and differentiation potential.

### 1.2 Applications

- Detachment of human pluripotent stem cell colonies and dissociation into cell clusters.
- Routine passaging of ES or iPS cell cultures.

### 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.
- Cell culture medium: StemMACS iPS-Brew XF (# 130-104-368).
- Cell attachment substrate, e.g., MACSmatrix Laminin 511 (# 130-136-454), vitronectin, or Corning® Matrigel®.
- 15 mL conical tubes.

## 2. Protocol

▲ The use of a small molecule ROCK inhibitor, such as StemMACS Y27632 or StemMACS Thiazovivin is essential to improve cell survival and attachment.

1. Aspirate the cell culture supernatant.
2. Wash the cell layer with 3 mL of buffer per well.
3. Add 1 mL of StemMACS Passaging Solution XF per well. Gently rock the plate to distribute the solution evenly.
4. 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 (figure 2).

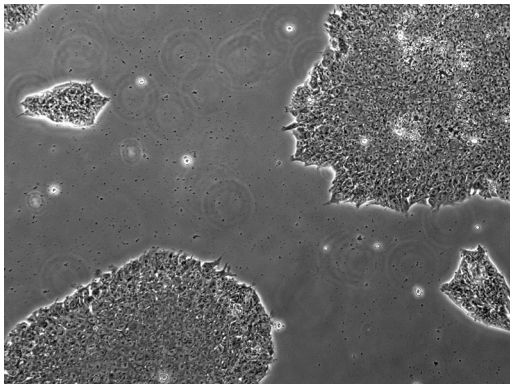


**Figure 1:** Colonies before addition of StemMACS Passaging Solution XF.

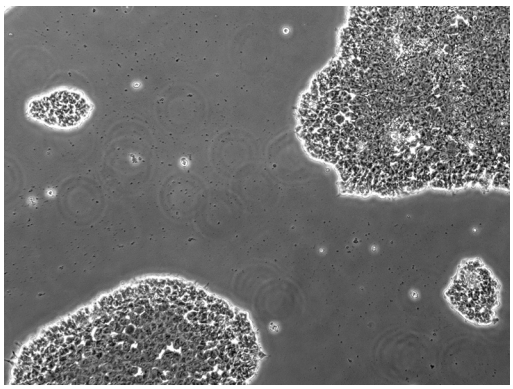


**Figure 2:** After 4 minutes incubation, colony edges start to lift off . At this point, the passaging solution should be removed.

5. Carefully remove the StemMACS Passaging Solution XF.
6. 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).


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7. Gently detach the colonies by rinsing the well with a 5 mL serological pipette.
8. Transfer the cell suspension into a 15 mL conical tube.
9. 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!
10. 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. Validated cell attachment substrates for use with StemMACS iPS-Brew XF include MACSmatrix Laminin 511, vitronectin, and Corning Matrigel.
11. After 48 hours, replace medium with fresh StemMACS iPS Brew XF without ROCK inhibitor.
12. 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).
13. Gently detach the colonies by rinsing the well with a 5 mL serological pipette.
14. Transfer the cell suspension into a 15 mL conical tube.
15. 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!
16. 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. Validated cell attachment substrates for use with StemMACS iPS-Brew XF include MACSmatrix Laminin 511, vitronectin, and Corning Matrigel.
17. After 48 hours, replace medium with fresh StemMACS iPS Brew XF without ROCK inhibitor.

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