

Contents

1. Description

1.1 Background information

1.2 Applications

1.3 Reagent requirements

2. Protocol

1. Description

This product is for research use only.

Components 100 mL StemMACS Passaging Solution XF, human

Specifications Osmolality: 260–300 mOsmol/kg
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., vitronectin, or 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).

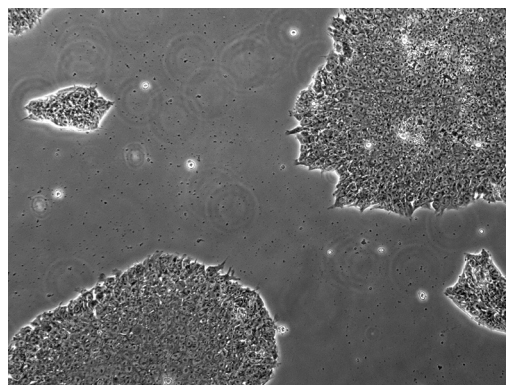


Figure 1: Colonies before addition of StemMACS Passaging Solution XF.

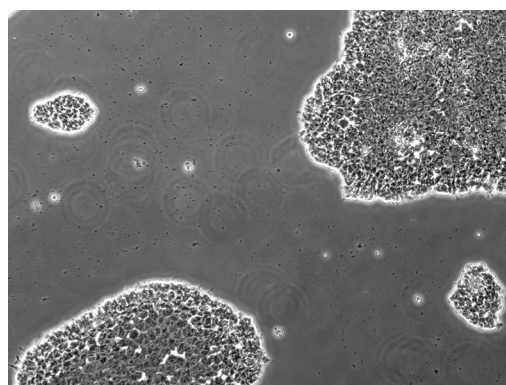


Figure 2: After 4 minutes incubation, colony edges start to lift off. At this point, the passaging solution should be removed.

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).

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 Matrigel® and vitronectin.

11. After 48 hours, replace medium with fresh StemMACS iPS Brew XF without ROCK inhibitor.
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).
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 Matrigel® and vitronectin.

11. After 48 hours, replace medium with fresh StemMACS iPS Brew XF without ROCK inhibitor.

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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

The Miltenyi Biotec logo and StemMACS are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Copyright © 2023 Miltenyi Biotec and/or its affiliates. All rights reserved.