Miltenyi Biotec

Protocol for MSC cultivation in MSC-Brew GMP Medium

Contents

1. Description

1.1 Purpose

This protocol describes the isolation of mesenchymal stem cells (MSCs) from human cell sources (like bone marrow, umbilical cord or adipose tissue) by plastic adherence and subsequent expansion.

1.2 Reagent and instrument requirements

- MSC-Brew GMP Basal Medium (# 170-076-315)
- MSC-Brew GMP Supplement R I (# 170-076-316)
- MSC-Brew GMP Supplement II (# 170-076-320)
- human TGF-β1 (e.g. Human TGF-β1, premium grade, # 130-095-067)
- Detachment enzyme (e.g. Trypsin based)
- Enzyme stop solution (e.g. Trypsin inhibitor)
- CliniMACS PBS/EDTA Buffer (# 700-25)
- Tissue culture vessel
- (Optional) CryoMACS DMSO 10 (# 170-076-303)
- (Optional) CryoMACS Freezing Bag, 50 – 1000 mL (e.g. CryoMACS Freezing Bag 50 # 200-074-400)
- (Optional) Trypan Blue Stain (Invitrogen, # 15250-061)
- CO_2 incubator, 37 °C with 5% CO_2 in air and >95% humidity
- Centrifuge
- Microscope
- (Optional) MACSQuant Analyser 10 (# 130-096-343)
- (Optional) Hemocytometer

2. Protocol

Isolation and expansion of human MSCs from primary tissue

MSCs are present at low frequencies in bone marrow samples as well as other tissues. This often necessitates their expansion.
The MSC-Brew GMP Medium is an optimized and standardized medium for the reproducible and reliable isolation and expansion of MSCs from human bone marrow and other human tissues, such as adipose tissue and umbilical cord.

2.1 Preparation of MSC-Brew GMP Medium

For preparation and usage of complete MSC-Brew GMP Medium please refer to the data sheet of MSC-Brew GMP Basal Medium 170-076-315, MSC-Brew GMP Supplement R I 170-076-316, and MSC-Brew GMP Supplement II 170-076-320.

2.2 Isolation of MSCs from primary tissue

▲ No coating is necessary.

▲ No additives are necessary.

1. Pre-warm supplemented MSC-Brew GMP Medium (referred as complete media) to 37 °C.
2. Prepare a suspension of human primary cells in MSC-Brew GMP Medium.
3. Determine cell number.
4. Transfer the appropriate amount of cells into a cell culture vessel using the appropriate cell density. An optimal cell density depending on the corresponding human tissue source can be found in table 1.

Human tissue source	Seeding density (cell number /cm^2)	Cell culture media (ml/cm^2)
Bone marrow mononuclear cells (BM MNC)	$1.6\times10^5/cm^2$	$0.2/cm^2$
Stromal vascular fraction (SVF)	$1\times10^5/cm^2$	$0.2/cm^2$
Cord blood (CB)	$1.6\times10^5/cm^2$	$0.2/cm^2$

Table 1: Optimal seeding density and cell culture media per cm^2 for cultivation of MSCs starting with primary tissue using MSC-Brew GMP Medium

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Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

5. Culture cells at 37 °C in an incubator with 5% CO_2 and >95% humidity.
6. Change complete media after 24 -48 hours.
7. Change medium every 4-5 days by removing medium completely from culture vessel and adding an appropriate amount of fresh complete MSC-Brew GMP Medium. Continue culturing the cells.
8. Check your cell culture under a microscope regularly. When MSCs have reached 80% confluency (presumably around day 10), proceed with Passaging of MSCs (see 2.3.)

2.3 Expansion and passaging of human MSCs

The following procedure can also be used for frozen MSCs as well as for switching a maintained MSCs culture into MSC-Brew GMP Medium. MSC can be transferred directly to MSC-Brew GMP Medium, without prior adaptation from any other culture media (including serum containing medium). It is recommended to seed MSCs with a density of 3×10^3 MSCs/cm^2.

1. Pre-warm detachment enzyme, CliniMACS PBS/EDTA Buffer and MSC-Brew GMP Medium to 37 °C.
2. Remove MSC-Brew GMP Medium from the tissue culture vessel.
3. Wash cells with CliniMACS PBS/EDTA Buffer to remove residual medium.
4. Add detachment enzyme to cover cells and incubate at 37 °C for 5–10 minutes.
5. Check under a microscope that MSCs are completely detached and dissociated. If cells are not fully detached, gently tap flask or increase incubation time for 2 – 5 minutes.

▲ **Note:** Good results were achieved using a Trypsin based detachment reagent.

6. Once MSCs are completely detached, add Trypsin Inhibitor and resuspend cells in MSC-Brew GMP Medium and transfer cell suspension into an appropriate vessel or bag.
7. Wash the cell culture vessel with an additional amount of MSC-Brew GMP Medium and add this solution into the appropriate vessel or bag.
8. Centrifuge cells at 300×g for 5-10 minutes at room temperature.
9. Remove supernatant and carefully resuspend cells in MSC-Brew GMP Medium.
10. Determine cell number and viability of cells (using for example a hemocytometer or MACSQuant Analyzer 10)
11. Plate 3×10^3 cells/cm^2 into new culture vessel with fresh MSC-Brew GMP Medium.
12. Culture cells at 37 °C in an incubator with 5% CO_2 and >95% humidity.
13. Check your cell culture under a microscope regularly. Before MSCs have reached 80% confluency, approx. after 2-4 days, repeat the passaging procedure.
14. MSC-Brew GMP Medium needs to be exchanged every 2-3 days by removing medium completely from culture vessel and adding an appropriate amount of fresh complete MSC-Brew GMP Medium.
15. Repeat expansion procedure until the desired number of cells is reached.

2.4. Cryopreservation of human MSCs

1. Rapidly resuspend MSC pellet with cold Freezing Solution.

▲ Note: Good results were achieved using 90% (recommended: $0.5\text{-}1.0\times10^6$ cells/ml; 1ml/vial) MSC-Brew GMP Medium (the addition of TGF-ß1 is not necessary for freezing) + 10% CryoMACS DMSO 10.

2. Immediately place the cryovials or cryobags in appropriate freezing container and place at –80 °C overnight.
3. Transfer the cryovials or cryobags into liquid nitrogen.

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