Miltenyi Biotec

CytoMix™ – MSC human

Order no. 130-093-552

Contents

1. Description

This product is for research use only.

Components	100 µg CytoMix – MSC, human
Quality control	Biological activity (proliferation assay using 3T3 cells): ≥ 8.0×10^5 IU/mg
Capacity	For the preparation of 1 L of supplemented MSC culture medium.
Product format	Lyophilized without carrier protein from a 0.2 µm filtered buffer solution.
Storage	Lyophilized CytoMix – MSC should be stored at –20 °C. The expiration date is indicated on the vial label. Upon reconstitution aliquots should be stored at –20 °C. Avoid repeated freeze-thaw cycles.

1.1 Background information

CytoMix – MSC, human is a composition of cytokines for the highly efficient and reproducible expansion of human multipotent mesenchymal stromal cells (MSCs) from a variety of tissues or isolated populations thereof. In combination with the StemMACS™ MSC Expansion Medium, human, CytoMix – MSC optimally supports the proliferation of human MSCs, especially after separation, for example, according to CD271 or MSCA-1 (W8B2) expression using MACS® Technology.

1.2 Applications

- Expansion of MSCs after separation according to specific markers.
- Expansion of MSCs from bone marrow mononuclear cells (BM MNCs) or other sources.

1.3 Reagent and instrument requirements

- StemMACS MSC Expansion Medium, human (# 130-091-680): optimized and standardized medium for the reproducible and reliable expansion of MSCs.
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.1% bovine serum albumin (BSA)
- Trypsin/EDTA (0.05%/0.53 mM)
- DMEM/20% FBS
- Trypan Blue stain and a hemocytometer
- Tissue culture flasks, e.g. T-25 tissue culture flask
- (Optional) StemMACS AdipoDiff Medium, human (# 130-091-677), StemMACS ChondroDiff Medium, human (# 130-091-679), or StemMACS OsteoDiff Medium, human (# 130-091-678): optimized differentiation medium for the generation of adipocytes, chondrocytes, or osteoblasts from human MSCs.
- (Optional) CD271 MicroBead Kit (APC), human (# 130-092-283), CD271 MicroBead Kit (PE), human (# 130-092-819), or Anti-MSCA-1 (W8B2) MicroBead Kit, human (# 130-093-583) to enrich for MSCs.

2. Protocol

2.1 Reconstitution

It is recommended to reconstitute lyophilized CytoMix – MSC with 1 mL of sterile 1× PBS buffer with 0.1% BSA.

2.2 Set-up of the expansion procedure

▲ Volumes given below are optimized for a starting cell number of up to 10^7 BM MNCs. When starting with different cell numbers, adjust volumes and growth size of tissue culture plate accordingly.

1. Pre-warm 5 mL of StemMACS MSC Expansion Medium per cell sample to 37 °C in a water bath or an incubator.
   ▲ **Note:** (Optional) Add 1% of penicillin-streptomycin to the StemMACS MSC Expansion Medium to prevent bacterial contamination of the cell culture.
2. When working with cells separated by MACS Technology, adjust the complete positive fraction to a final volume of 5 mL with StemMACS MSC Expansion Medium.
   When working with BM MNCs, adjust 10^7 BM MNCs to a final volume of 5 mL with StemMACS MSC Expansion Medium.
3. Transfer the diluted cell sample to a T-25 tissue culture flask. For different starting cell numbers choose a vessel according to the table in section 2.2.
4. Culture cells at 37 °C in an incubator with 5% CO_2 and >95% humidity.

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5. Examine the cell culture plate every two days. Singularize the MSCs when colonies are formed as described in 3.1.
   ▲ **Note:** When colonies are too dense, MSCs could differentiate spontaneously.
6. Continue culturing cells at 37 °C in an incubator with 5% CO_2 and >95% humidity.
7. Change StemMACS MSC Expansion Medium weekly. Remove StemMACS MSC Expansion Medium completely from the T-25 tissue culture flask and add 5 mL of fresh StemMACS MSC Expansion Medium. Continue culturing cells.
8. Check your cell culture under a microscope regularly. When MSCs have reached 80% confluency (presumably at day 12), proceed with 2.2.
   ▲ **Note:** In some cases, MSCs are distributed unevenly over the growth area. Thus, certain colonies or areas reach 80% confluency earlier than others. A protocol to equally distribute the cells for further cultivation can be found in 3.1.

2.3 Passaging of human multipotent mesenchymal stromal cells (MSCs)

1. Pre-warm Trypsin/EDTA (0.05%/0.53 mM), PBS and StemMACS MSC Expansion Medium to 37 °C in a water bath or an incubator.
2. Remove StemMACS MSC Expansion Medium completely from the tissue culture flask.
3. Wash cells with 5 mL of PBS to remove residual StemMACS MSC Expansion Medium.
4. Add a volume Trypsin/EDTA (0.05%/0.53 mM) to cover cells and incubate at 37 °C for 5–10 minutes (see table below).

Vessel	Growth surface (cm^2)	Total cell number ($\times 10^5$)	Trypsin/EDTA volume (mL)	DMEM/FBS volume (mL)	StemMACS MSC Expansion Medium volume (mL)
6-well plate	9.6	0.6–0.8	0.5	6	2
T25	25	1.5–2.0	2	10	5
T75	75	4.5–6.0	5	20	15
T175	175	10.0–14.0	5	20	25

5. Check under a microscope that MSCs are completely dissociated. If not, gently tap plate or increase the incubation time for a few more minutes to facilitate dissociation of the cells.
   ▲ **Note:** Time of trypsination may vary, but usually cells dissociate within 5 to 15 minutes.
6. Once MSCs are completely detached, add a volume of DMEM with 20% FBS (see table above), resuspend cells by pipetting and transfer them to a 15 mL conical tube.
7. Wash the culture plate with an additional volume DMEM with 20% FBS and collect all cells in the 15 mL conical tube.
8. Centrifuge cells at 300×g for 10 minutes at room temperature.
9. Remove supernatant and carefully resuspend cells in 5 mL of StemMACS MSC Expansion Medium.
10. Determine cell number and viability using a hemocytometer by Trypan Blue exclusion.
11. Choose a cell culture flask according to the cell number determined and choose the volume of StemMACS MSC Expansion Medium for cultivation (see table above).
    ▲ **Note:** Cell numbers of harvested cells should be adjusted to 6–8×10^3 MSCs/cm^2 growth surface.
12. In order to prepare StemMACS MSC Expansion Medium-CytoMix – MSC, thaw sufficient CytoMix – MSC to dilute 1 µL for every 1 mL StemMACS MSC Expansion Medium used.
    ▲ **Note:** (Optional) Add 1% penicillin-streptomycin to the StemMACS MSC Expansion Medium to prevent bacterial contamination of the cell culture.
13. Transfer cells suspended in StemMACS MSC Expansion Medium-CytoMix – MSC to the appropriate cell culture flask (see table above).
14. Culture cells at 37 °C in an incubator with 5% CO_2 and >95% humidity.
15. Check your cell culture under a microscope regularly. Before MSCs have reached 80% confluency, approx. after 2–3 days, repeat the passaging procedure. If confluency has not reached 80% after 2–3 days change the StemMACS MSC Expansion Medium-CytoMix – MSC.
16. Repeat expansion procedure until a sufficient number of MSCs is reached.
    ▲ **Note:** The number of passages that can be achieved while maintaining the full differentiation potential of MSCs is donor-dependent. We recommend not to exceed 4 passages.
    ▲ **Note:** It is possible that MSCs spontaneously differentiate into, e.g., osteoblasts. This mainly occurs when MSCs are too dense. If this occurs, split cells before 80% confluency is reached.
    ▲ **Note:** For freezing and thawing protocols for MSCs see sections 3.2 and 3.3, respectively.
17. (Optional) Differentiate cells with StemMACS AdipoDiff Medium, StemMACS ChondroDiff Medium, or StemMACS OsteoDiff Medium.
    ▲ **Note:** For protocols describing the differentiation of human MSCs, please refer to the protocol in the tab "Resources" at www.miltenyibiotec.com/130-093-552.

3. Appendix

3.1 Redistribution of unevenly populated tissue cultures

1. Pre-warm Trypsin/EDTA (0.05%/0.53 mM), PBS, and StemMACS MSC Expansion Medium to 37 °C in a water bath or incubator.
2. Remove StemMACS MSC Expansion Medium from the tissue culture flask.
3. Wash cells with PBS to remove residual StemMACS MSC Expansion Medium.
4. Choose an appropriate volume of Trypsin/EDTA (0.05%/0.53 mM) to cover cells and incubate at 37 °C for 5–10 minutes (see table below).

Vessel	Growth surface (cm^2)	Total cell number ($\times 10^5$)	Trypsin/EDTA volume (mL)	StemMACS MSC Expansion Medium volume (mL)
6-well plate	9.6	0.6–0.8	0.2	2
T25	25	1.5–2.0	0.5	5

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5. Check under a microscope that MSCs are completely dissociated. If not, gently tap flask or increase the incubation time for some more minutes to facilitate dissociation of the cells.
   ▲ **Note:** Time of trypsination may vary, but usually cells dissociate within 5 to 15 minutes.
6. Once MSCs are completely detached, add appropriate volume StemMACS MSC Expansion Medium with 1 mL CytoMix – MSC per 1 mL of medium (see table above) and resuspend cells by pipetting.
7. Continue culturing the cells.

3.2 Freezing of multipotent mesenchymal stromal cells (MSCs)

3.2.1 Reagent and cell culture requirements

- Dimethyl sulfoxide (DMSO)
- Cryogenic storage vials
- Box suitable for storage in liquid nitrogen
- 50 mL conical tubes
- PBS/EDTA buffer (PBS with 2 mM EDTA), pH 7.2
- Freezing medium:
  supplement StemMACS MSC Expansion Medium with:
  10% FCS
  10% DMSO
  1% penicillin-streptomycin

3.2.2 Protocol

1. Pre-cool freezing medium on ice.
2. Harvest cells.
3. Resuspend cells carefully in freezing medium at a concentration of 5×10^5 cells/mL.
4. Immediately aliquot 1.8 mL of cells into appropriate cryogenic storage vials and close the lid tightly.
5. Place the vials in the pre-cooled box and directly store at –70 °C.
6. After 24 hours, transfer the frozen cryogenic storage vials to an ultra-low temperature freezer (–150 °C) or to liquid nitrogen for long-term storage.

3.3 Thawing of multipotent mesenchymal stromal cells (MSCs)

▲ Perform all of the following steps under sterile conditions in a laminar flow hood.

1. Fill 50 mL conical tubes with water. Pre-warm to 37 °C.
2. Place frozen vials into 50 mL conical tubes containing warmed water. Do not close the lid of the conical tube.
3. After thawing, transfer cells to a sterile 50 mL conical tube.
4. Wash cells by adding 20 mL of PBS/EDTA buffer, mix gently, and centrifuge at 300×g for 10 minutes at room temperature.
5. Aspirate supernatant completely.
6. Resuspend cells carefully in the appropriate medium (e.g. StemMACS MSC Expansion Medium or the preferred StemMACS differentiation medium).

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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