Product Sheet Research Grade MSCs

Product Information

Product name	Human bone marrow-derived MSC
Quality grade	Research grade
Passage	3

Analytical Methods – Quality Control

Analysis	**Methods**
MSC surface marker expression[1]	Flow cytometry – CD73+, CD90+, CD105+
Contaminating cells[1]	Flow cytometry – CD14-, CD31-, CD34-, CD45-
Viability[1]	Cell calculation
Morphology	Visual inspection of cell culture, microscopy
Sterility	Ph Eur 2.6.1 Direct inoculation
Mycoplasma	Ph Eur 2.6.7, PCR method
Endotoxin	Ph Eur 2.6.14 Gel-clot

1. Cryopreserved and thawed samples

Storage

Storage condition	Vapor phase nitrogen
Shelf life	2 years

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Donor Information

Cellcolabs' hBM-MSCs are manufactured from bone-marrow obtained from healthy donors 18-28 years old.

Donors are screened for the following infectious diseases:

- Malaria
- Syphilis
- Tuberculosis
- Brucellosis
- West Nile Virus
- Lurs
- Hepatitis B
- Babesiosis
- Creutzfeldt Jakob
- HIV 1/2
- HCV
- HTLV 1/2
- Leishmaniasis
- Lebra
- Typhus fever
- Trypanosoma Cruzi
- Chronic Lyme disease
- Chronic Q-fever

Donors are screened through physical examination and questionnaire for general health status, various other diseases, and medications.

Donors are tested for general haematology and biochemistry.

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Expansion Protocol Example

Summary
This protocol describes a general procedure for thawing, washing and seeding Cellcolabs' hBM-MSC.

1. Media preparation and warming

1.1 To optimize the expansion capacity of Cellcolabs' hBM-MSC, Dulbecco's Modified Eagle Medium (DMEM) with 5% of human platelet lysate (hPL) is recommended by our scientists. Please consult Cellcolabs if you would like to find the optimal solution for your media.

1.2 The medium should be well mixed and pre-warmed in the water bath at 37 °C for at least 30 minutes. After usage, the medium should be stored in the fridge between 2-8 °C for future use.

2. Thawing and washing

2.1 Thaw Cellcolabs' MSC cryotube in 37 °C water bath until chunk of ice is visible (around 2-3 minutes).

2.2 Transfer all contents in Cellcolabs' MSC cryotube into a 15 ml tube immediately after thawing in a biosafety cabinet.

2.3 Add 10 ml medium into the tube and suspend the content in the tube.

2.4 Set the centrifuge at 500 g, 7 minutes and at room temperature.

2.5 Discard the supernatant by pipette and re-suspend the pallet in 1 ml complete medium.

2.6 Count the cell number and seed the cells in culture flasks, the density of cells is recommended between 3500-4000 cells per cm^2.

2.7 For example, prepare a 175 cm^2 flask, add 25ml medium into the flask.

2.8 Incubate the cells in the incubator at 37 °C, 5% CO2, 85-95% humidity.

2.9 Visual inspection of the cell culture shall be performed every third day, including observing overgrowth, microbial contamination, and morphology.

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3. Media exchanging

3.1 First medium exchange shall be performed 3-4 days after initial plating of the cells.

3.2 Pre-warm the completed medium in the water bath at 37 °C for at least 30 minutes.

3.3 Discard the medium by aspiration. Do not disturb the cell layer during aspiration.

3.4 Add 25 ml pre-warmed complete medium carefully into the flask.

3.5 Incubate the cells in the incubator at 37 °C, 5% CO2, 85-95% humidity.

4. Cell expanding

4.1 Once the MSCs is observed more than 70% confluent but not 100%, the cell expanding shall be performed.

4.2 Aspirate and discard the old medium in the flask.

4.3 Add 10 ml PBS into the flask. Do not flush direct toward the cell layer to avoid damaging cells.

4.4 Gently rinse the cell layer.

4.5 Aspirate and discard PBS.

4.6 Add dissociation reagent 5ml/175cm^2, make sure the reagent covers the whole area of the cell layer.

4.7 Incubate at 37 °C until cells have detached, approx. 5-10 min (For 175 cm^2 flask).

4.8 To ensure complete cell detachment from the surface check the progress of the detachment by examining the flasks under the microscope. Gently tap flask to dislodge cells if necessary.

4.9 Add 10 ml complete medium to quench.

4.10 Use a pipette to flush the cell surface to detach all cells.

4.11 Transfer the cells to a 50 ml tube.

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4.12 If necessary, use PBS to flush remaining cells in the flask, and transfer to the same 50 ml tube.

4.13 Set the centrifuge at 500 g, 7 minutes and at room temperature.

4.14 Discard the supernatant and resuspend cell pellet with 1-5 ml complete medium.

4.15 Count the cells in a haemocytometer or automated cell counters.

4.16 Seed the cells into multiple flasks with recommended density (3500-4000 cells per cm^2)

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