

User's Manual and Instructions

Cryopreserved mMSC

Catalog number: Z7030061

Introduction:

Mesenchymal Stem cells (MSCs) are also called multipotent stromal cells. They are fibroblast-like Stem/progenitor cells residing in different tissues, including bone marrow and adipose tissue (Pittenger et al., 1999; Miyahara et al., 2006). Since MSCs can not only differentiate into different cell types (cartilage, muscle, endothelial and neural cells) but also secreting soluble factors to alter the tissue microenvironment (Pittenger et al., 1999; Dezawa et al., 2005; Muguruma et al., 2006), human MSCs have been used in clinical trials to treat various human diseases including: graft-versus-host disease (GVHD), cardiovascular diseases, neurological and inherited diseases (Lazarus et al., 2005; Perin et al., 2004; Wollert et al., 2004; Mazzini et al., 2003). It is generally accepted that mouse bone marrow derived MSCs are CD44+, Sca1+, CD45-, c-Kit-, CD34- and CD31- (Gnecchi et al., 2009; Nadri et al. 2007).

Specification and Characterization:

BioChain's mouse MSCs were derived from the bone marrow of 7-8 week old C57/Bl6 mice and were selected by the attachment to culture vessels. Our mMSC product is delivered at the 4-5th passage as cryopreserved cells. Each cryovial contains $>5 \times 10^5$ cells in 1 ml freezing medium. They can be maintained in BioChain's mouse MSC growth medium and differentiate into different cell types upon induction.

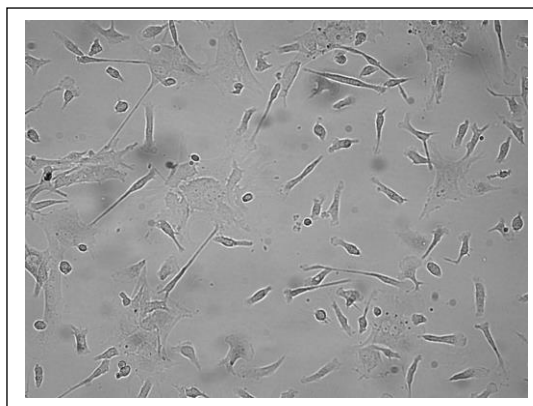


Figure 1. Morphology of mouse MSCs (passage 4) in culture. 100x.

Shipping

Cryopreserved cells are shipped on dry ice.

Instructions for culturing MSC

I. Preparation of mMSC Growth Medium

We recommend the use of BioChain's mMSC growth medium (Cat# Z7030063) for culturing our mMSC.

1. Thaw mMSC growth medium supplement (Cat# Z7030065) in a water bath at room temperature.
2. Prepare mMSC growth medium by adding the entire volume (35 ml) of MSC growth medium supplement to the bottle (500 ml) of mMSC basal medium (Cat# Z7030064). BioChain's mMSC growth medium does not contain antibiotics, but antibiotics may be added to the medium if contamination is a concern.
3. Prior to use, warm up a portion of the mMSC growth medium in a 37°C water bath.

II. Thawing frozen cells

1. Warm MSC growth medium in a 37°C water bath.
2. Wipe the outside of the frozen vial with 70% ethanol. Quickly thaw the frozen cells in the water Bath at 37°C.
3. Aseptically transfer the cell suspension to a 15 ml tube. Rinse the vial with 1 ml of growth medium; and combine the rinse with the cells in 15 ml tube. Centrifuge at 170g (or 1400rpm) for 5 minutes to precipitate the cells. Remove the supernatant. Add 5 ml of fresh mouse MSC medium and put into a T25 flask.
4. Incubate the cells at 37°C with 5% CO₂ and 95% air in a humidified incubator. Change medium every other day. A healthy culture displays a fibroblast-like morphology and cell number should double after two to three days in culture.

III. Sub-culturing cells

1. Subculture the cells when they reach 90% confluence.
2. Change medium the day before passing the cells.
3. Warm Dulbecco's PBS, 0.05% trypsin/EDTA and mMSC growth medium in a 37°C water bath.
4. Rinse the cells with Dulbecco's PBS.
5. Incubate the cells with trypsin/EDTA solution (1.5 ml/25 cm²) 3-5 minutes until approx. 90% of the cells begin to detach. Pepping and gently padding the vessel to detach the cells.
6. Add fetal bovine serum equal to 1/10th volume of the trypsin/EDTA to neutralize trypsin, gently shake the culture vessel to mix.
7. Gently re-suspend the cells and transfer the cells into a 15 ml conical tube.
8. Centrifuge the cell suspension at 170 x g for 5 minutes at room temperature.
9. Carefully remove the supernatant without disturbing the cell pellet. Re-suspend the cells in growth medium.
10. Plate them in new culture vessels in 1:4 ratio.
11. Incubate the cells at 37°C with 5% CO₂ and 95% air in a humidified incubator. Change medium every other day.

Related Products

- mMSC Growth Medium (Cat#: Z7030063)
- mMSC Basal Medium (Cat#: Z7030064)
- mMSC Growth Medium Supplement (Cat#: Z7030065)
- mMSC Freezing Medium (Cat#: Z7030066)

Reference:

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