NEUROMICS

Human Bone Marrow-Derived Mesenchymal Stem Cells

Catalog Number	MSC001
Storage	Liquid Nitrogen
Cell Number:	Frozen Vial (> 5 x 105cells/vial)
Viablitliy	≥70% when thawed

Caution: Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when handling these materials. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination. The listed dilutions are for recommendation only and the ender users should optimize the final conditions.

General Information

HBMMSCs are isolated from human Bone Marrow tissues and demonstrated with spindle-shaped, and fibroblast-like cells. Each lot is tested to ensure the cells can be passaged at least three times (i.e., approximately 9 to 10 population doublings) after thaw in complete growth media (Mesenchymal Stem Cell Growth Medium (MSCGM), Alpha-35), when cultured following the detailed protocol described below).

Characterization of the cells:

Positive for CD29, CD44, CD73, CD90, CD105, and CD166 (greater than 95% of the cell population expresses these markers by flow cytometry).

Negative for CD14, CD31, CD34, and CD45 (less than 2% of cell population expresses these markers by flow cytometry).

Product Use: HBMMSCss are for research use only.
Shipping: Shipping on dry ice or in LN2 is required.

Handling of Arriving Cells

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80ºC freezer for short period storage or a liquid nitrogen tank for long term storage. Thaw the cells in a 37°C water bath, and then quickly transfer the cells into a T75 flask with 15 ml MSCGM and incubated overnight in a 37 ºC CO2 incubator and change the medium next day (15 ml complete MSCGM) and every other thereafter.

Subculture Protocol

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

> HBMMSCs are contact inhibited. It is essential that the cells be subculture BEFORE reaching confluence as post-confluent cells exhibit changes in morphology, slower proliferation, and reduced differentiation capacity after passaging.

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1. Rinse the cells in T75 flask with 15ml HBSS (Room Temperature, RT) twice.
2. Add 4ml of Trypsin/EDTA (RT) (cAP-23) into one T75 flask (make sure the whole surface of the T75 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution within 30 seconds with aspiration.
3. Leave the T75 flask with the cells at RT for 1 minute (the cells usually will detach from the surface within 1-2 minutes). You can monitor the cells under microscope and when most of cells become rounded up, hit the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.
4. Add 10ml Trypsin Neutralization Buffer and spin the cells down with 800g for 5 minutes.
5. Re-suspend the cell pellet with 30 – 45 ml of MSCGM and the cell suspension is transferred directly into 2 or 4 pre-coated T75 flasks (15ml each, and the cells are sub-cultured at 1:2 or 1:3 ratios)
6. Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:3 ratio).

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