

Human Mesenchymal Stem Cells (hMSCs) Serum-Free Medium for cell proliferation suppression

Xyltech™ MSC-02 Animal-Free

Catalog Number (BBARL/NIPRO): 10402/87-334 100 mL

1. Product features

This product is a culture medium suitable for suppressive growth control of human mesenchymal stem cells (hMSCs). Xyltech™ MSC-02 Animal-Free can be used to control the growth rate of hMSCs in combination with Xyltech™ Growth MSC, which is a serum-free medium for hMSCs proliferation. This product is an *Animal-Free culture medium. In addition, there is no need to pre-coat culture vessels with any type of attachment matrix.

*Animal-Free: It contains no ingredients derived from animals.

2. Precautions for use

Xyltech™ MSC-02 Animal-Free does not contain substances that neutralize trypsin activity. When subculturing cells with trypsin, it is strongly recommended that the trypsin activity be sufficiently neutralized with a trypsin inhibitor. Dilution washing alone does not completely remove trypsin activity and the remaining protease activity will reduce subsequent cell growth.

This product is a research reagent. It cannot be used for human or animal treatment or diagnostic purposes.

3. Storage

Store Xyltech™ MSC-02 Animal-Free in a freezer (-20°C). Do not refreeze the thawed medium to avoid deterioration of some active ingredients.

4. Thawing and preparation

This product should be thawed in a **37°C water bath**, being careful not to submerge the entire bottle. After thawing, use it shortly.

Fine particles may be observed but there is no influence on quality.

5. Example of cell culture protocol for normal human adipose-derived stem cells (ADSCs) culture using Xyltech™ MSC-02 Animal-Free and Xyltech™ Growth MSC (AF)

5-1. Cells and reagents

- | | |
|---|----------------------|
| • Normal human ADSCs (100 mm-dish) | |
| • Xyltech™ MSC-02 Animal-Free (growth suppression medium) | *This product |
| • Xyltech™ Growth MSC Medium | (BBARL CAT. #10411) |
| • Xyltech™ Growth MSC Supplement AF | (BBARL CAT. #10413) |
| • r-TE (Recombinant Trypsin/EDTA Solution) | (NIPRO CAT. #87-974) |
| • s-TI (Synthetic Trypsin Inhibitor Solution) | (NIPRO CAT. #87-975) |
| • D-PBS (-) | |

5-2. Growth control of normal human ADSCs

1. Before using Xyltech™ Growth MSC (AF), add the entire amount of Xyltech™ Growth MSC Supplement AF (thawed before use) to Xyltech™ Growth MSC Medium and mix well.

2. Warm the Xyltech™ Growth MSC (AF), D-PBS (-), r-TE, and s-TI in a 37°C water bath.
3. Remove the culture supernatant of selected normal human ADSCs that have reached approximately 80% confluence (sub confluence).
4. Rinse the cell layer with 5 mL of D-PBS (-).
5. Add 0.5 mL of r-TE and incubate at 37°C for approximately 2 minutes.
6. Add 0.5 mL of s-TI, mix well, gently pipette up and down several times, collect cells from the dish, and centrifuge at 1,000 rpm, for 5 minutes.
7. Aspirate the supernatant and add the appropriate amount of Xyltech™ Growth MSC (AF) to resuspend the cells and seed into a new tissue culture dish.
8. The next day, use a phase-contrast microscope to confirm that the cells have engrafted and replace the growth medium with pre-warmed Xyltech™ MSC-02 Animal-Free culture medium.
9. The cells can be cultured for up to 3 days with Xyltech™ MSC-02 Animal-Free. The cells start to regrow quickly and become confluent 1-2 days after changing the medium back to the Xyltech™ Growth MSC (AF). Begin subculture and/or experiments with the cells.

5-3. Phase contrast microscope images of normal human ADSCs cultured with Xyltech™ Growth MSC (AF) or Xyltech™ MSC-02 Animal-Free

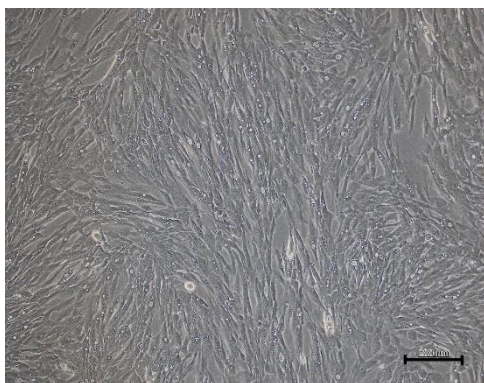
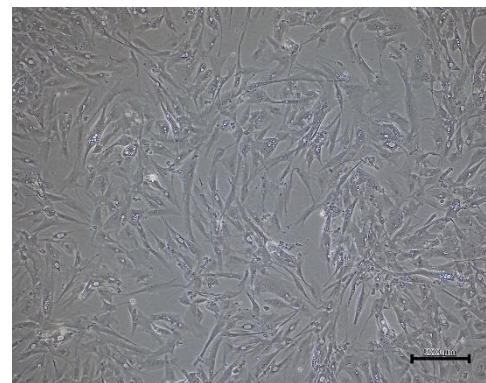


Fig. 1 Normal human ADSCs cultured with Xyltech™ Growth MSC (AF) for 3 days.



Bars=200 μm

Fig. 2 Normal human ADSCs cultured with Xyltech™ MSC-02 Animal-Free (growth suppressive medium) for 3 days.

*The protocol is based on experimental results. It may be necessary to adjust seeding density, and passage timing according to the cells. This protocol is intended for research purposes only.

6. For Inquiries about products

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