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

# Xyltech<sup>™</sup> MSC-01 Xeno-Free

Catalog Number (BBARL/NIPRO): 10401/87-330 100 mL

# 1. Product features

This product is a culture medium suitable for suppressive growth control of human mesenchymal stem cells (hMSCs). Xyltech<sup>™</sup> MSC-01 Xeno-Free can be used to control the growth rate of hMSCs in combination with Xyltech<sup>™</sup> Growth MSC, which is a serum-free medium for hMSCs proliferation. This product is \*Xeno-Free culture medium. In addition, there is no need to pre-coat culture vessels with any type of attachment matrix.

\*Xeno-Free: It contains human-derived components. Any other animal-derived component is free.

## 2. Precautions for use

Xyltech<sup>™</sup> MSC-01 Xeno-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<sup>™</sup> MSC-01 Xeno-Free in a freezer (-20°C). After thawing, store in a cool, dark place (2-8°C). Also, do not refreeze the thawed medium to avoid deterioration of some active ingredients.

## 4. Thawing and preparation

This product should be thawed at **room temperature (20-25°C)**. Avoid thawing in a water bath. After thawing, store the medium in a cool, dark place (2-8°C) and use within one month. <u>Fine particles may be observed but there is no influence on quality.</u>

5. Example of cell culture protocol for normal human adipose-derived stem cells (ADSCs) culture using Xyltech™ MSC-01 Xeno-Free and Xyltech™ Growth MSC (Xeno-Free)

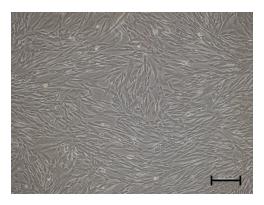
# 5-1. Cells and reagents

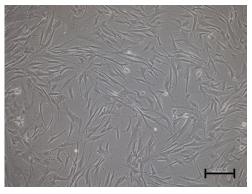
- Normal human ADSCs (100 mm-dish)
- Xyltech™ MSC-01 Xeno-Free (growth suppression medium) \*This product
- Xyltech<sup>™</sup> Growth MSC Medium
  Xyltech<sup>™</sup> Growth MSC Supplement XF
  r-TE (r-Trypsin/EDTA Solution)
  s-TI (Synthetic Trypsin Inhibitor Solution)
  D-PBS (-)
  (BBARL CAT. #: 10411)
  (BBARL CAT. #: 10412)
  (NIPRO CAT. #: 87-974)
  (NIPRO CAT. #: 87-975)

#### 5-2. Growth control of normal human ADSCs

- 1. Before using Xyltech<sup>™</sup> Growth MSC, add the entire amount of Xyltech<sup>™</sup> Growth MSC Supplement XF (thawed before use) to Xyltech<sup>™</sup> Growth MSC Medium and mix well.
- 2. Warm the Xyltech<sup>™</sup> Growth MSC, 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 around 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<sup>™</sup> Growth MSC to resuspend the cells and seed into a new tissue culture dish.

- 8. The next day, using a phase-contrast microscope to confirm the cells are engrafted and replace the growth medium with the Xyltech<sup>™</sup> MSC-01 Xeno-Free culture medium.
- 9. The cells can be cultured for up to 3 days with Xyltech<sup>™</sup> MSC-01 Xeno-Free. The cells start to regrow quickly and become confluent 1-2 days after changing the medium back to the Xyltech<sup>™</sup> Growth MSC. Begin subculture and/or experiments with the cells.
- 5-3. Phase contrast microscope images of normal human ADSCs cultured with Xyltech™ Growth MSC (Xeno-Fee) or Xyltech™ MSC-01 Xeno-Free





Bars=200 µm

Fig. 1 Normal human ADSCs cultured with Xyltech<sup>™</sup> Growth MSC (Xeno-Free) for 3 days.

Fig. 2 Normal human ADSCs cultured with <u>Xyltech™ MSC-01 Xeno-Free</u> (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 Bourbon Biomedical Advanced Research Laboratories, Inc. (BBARL, Inc.) 1-3-1, Ekimae, Kashiwazaki-city, Niigata, TEL: +81-257-23-2769 E-mail: support@bourbon-barl.co.jp URL: https://www.bourbon-barl.co.jp/eg/

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