BBARL, Inc.

Human Mesenchymal Stem Cell (hMSC) Serum-Free Medium for cell proliferation suppression

Xyltech™ BMT-01

Catalog Number (BBARL/NIPRO): 10201/87-283 100 mL

1. Product features

This product is a synthetic culture medium suitable for suppressive growth control of human mesenchymal stem cell (hMSC). Xyltech™ BMT-01 can be used to control the growth rate of hMSC in combination with Xyltech™ Growth BMT, which is a serum-free medium for hMSC proliferation. This product is serum-free culture medium.

2. Precautions for use

Xyltech™ BMT-01 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™ BMT-01 in a cool, dark place (2-8°C). Do not freeze the medium to avoid deterioration of some active ingredients.

4. Example of suppressive growth control protocol for normal human adipose-derived stem cells (ADSCs) culture using Xyltech™ BMT-01

4-1. Cells and reagents

- Normal human ADSCs (100 mm-dish, Fibronectin coated)
- Xyltech™ BMT-01 (growth suppression medium) ***This product**
- Xyltech™ Growth BMT (growth medium) (CAT. # (BBARL/NIPRO): 10211/87-286)
- r-TE (r-Trypsin/EDTA Solution) (NIPRO CAT. #: 87-974)
- s-TI (Synthetic Trypsin Inhibitor Solution) (NIPRO CAT. #: 87-975)
- D-PBS (-)

4-2. Growth control of normal human ADSCs

1. Warm the culture medium, D-PBS (-), r-TE, and s-TI in a 37°C water bath.
2. Remove the culture supernatant of selected normal human ADSCs that have reached around 80% confluence (sub confluence).
3. Rinse the cell layer with 5 mL of D-PBS (-).
4. Add 0.5 mL of r-TE and incubate at 37°C for approximately 2 minutes.
5. 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.

6. Aspirate the supernatant and add the appropriate amount of Xyltech™ Growth BMT medium to resuspend the cells and seed into a fibronectin coated new tissue culture dish.
7. The next day, use a phase-contrast microscope to confirm the cells are engrafted and replace the growth medium with the Xyltech™ BMT-01 culture medium.
8. The cells can be cultured up to 3 days with Xyltech™ BMT-01. The cells start to regrow quickly and become confluent 1-2 days after changing the medium back to the Xyltech™ Growth BMT medium. Begin subculture and/or experiments with the cells.

4-3. Phase contrast microscope images of normal human ADSCs cultured with Xyltech™ Growth BMT or Xyltech™ BMT-01

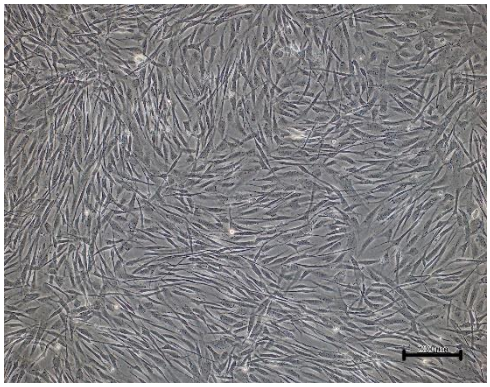
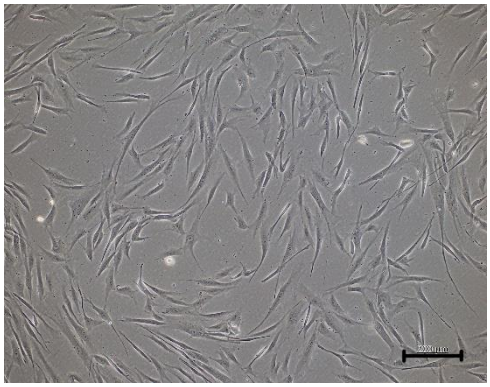


Fig. 1 Normal human ADSCs cultured with Xyltech™ Growth BMT for 3 days.



Bars=200 μm

Fig. 2 Normal human ADSCs cultured with Xyltech™ BMT-01 (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.

5. For Inquiries about products

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