R:STEM Medium for hMSC High Growth

User Guide & Product Information

Product Description:

R: STEM Medium for hMSC High Growth is a medium made by utilizing the know-how conducted by ROHTO Pharmaceutical Co., Ltd., Japan. It is manufactured under the same raw material grade, manufacturing environment, and quality control as the media that is used in our clinical trials. This medium is a serum-free medium that does not contain any animal-derived or human-derived materials such as serum, which could date back to the secondary materials, and this medium is composed of chemically defined components. Therefore, this medium is designed to keep batch-to-batch consistency to obtain highly reproducible. It is also considered to have low risks of contamination by viruses, etc., because the medium does not use animal-derived or human-derived materials. R: STEM Medium for hMSC High Growth is suitable for cell isolation, primary cell culture and cell expansion of mesenchymal stromal cells (hMSC). If you use the recommended flasks, it is no need to pre-coat flasks, and this medium is ready for use without adding serum or other serum substitute. R: STEM Medium for hMSC High Growth has been used in the culture of umbilical cord-derived hMSC, adipose-derived hMSC, and bone marrow-derived hMSC.

Storage:

Please store in a dark place below -30 ° C and use within the expiration date.

Avoid refreezing since it will deteriorate once thawed.

Thawing and Usage:

Please thaw this medium at room temperature (15-25 ° C).

After thawing, mix gently and immediately store in a dark place near 2-6 ° C.

Thawed medium should be used up within one month after thawing.

When using in small amounts, aseptically separate the required amount, bring it to room temperature, and then add it to the cells.

After thawing, some of the media components may be confirmed as white precipitates, but it has been confirmed to be no problem with the quality of medium.

Caution: Do not heat above room temperature with a heater when thawing. It causes denaturation of medium components.

Recommended Flask:

Corning® CellBIND surface flask is recommended to be used without adhesion molecules precoating.

Recommended Protocols:

I. hMSC cell isolation and primary culture

1. Adipose tissue is dispersed by collagenase to obtain a cell liquid containing hMSC.
2. Add the culture medium (more than twice the amount of cell liquid) into cell liquid and collect it into a 50 mL centrifuge tube, and centrifuge (room temperature, 800 G, 5 minutes or longer).
3. Carefully remove the supernatant, add 45 mL of the medium and suspend well.
4. Centrifuge (room temperature, 400 G, 5 minutes or longer) and then carefully remove the supernatant.
5. Add 30 mL of the medium again and suspend well.
6. After centrifugation (room temperature, 400 G, 5 minutes or longer), carefully remove the supernatant.
7. Add an appropriate amount of medium, suspend well and then seed cells on the Corning® CellBIND surfaced flasks (recommended flask).

(Recommended medium amount: 10 mL or more per 75 cm^2 seeding area)

8. Incubate at 37 ° C, 5% CO_2 incubator.
9. Observe cell conditions and subculture when it reaches 70-90% confluence.
10. If the cell culture continues for more than 4 days, change the medium for every 3 days.

Caution: Since optimal culture conditions depend on different tissue types, this is for reference only. In addition, this medium does not contain antibiotics. Add antibiotics if it is needed.

II. Cell passage and expansion

1. Wash the cells with PBS when it reaches to 70-90% confluence on Corning® CellBIND flask.
2. Carefully remove the washing solution PBS and then detach the cells with cell dissociation solution e.g. trypsin.
3. Transfer the entire amount of cell liquid into a centrifuge tube.
4. Add the same amount of medium as the cell liquid to the flask and wash the entire flask.
5. Collect the whole liquid to the same previous cell liquid-containing centrifuge tube.
6. Centrifuge the cell liquid (room temperature, 400 G, 5 minutes) and then carefully remove all supernatants.
7. Add 10 mL or more of the medium and mix well.
8. Centrifuge again and carefully remove the supernatant.
9. Add necessary amount of medium and adjust cell density to around 1.0 x 10^5 to 10^6 cells / mL, and

count cell numbers. Adjust the cell seeding density to 5000-10000 cells / cm^2.

10. Suspend cell liquid well and seed cells on the Corning® Cell BIND flasks.

11. Incubate at 37 ° C, 5% CO_2 incubator.

12. Observe cell conditions and subculture when it reaches 70-90% confluence.

13. If the cell culture continues for more than 4 days, change the medium for every 3 days.

Caution: Avoid over confluency of cells.

Particularly, umbilical cord-derived hMSCs may be difficult to be detached, so it is strongly recommended to passage the cells within 4 days culture.

Since this medium is a serum-free medium and does not contain trypsin inhibitors, if the dissociation reagent remains, it will affect the culture performance. Carefully do the washing steps so that the dissociation agent does not remain.

II. Seeding from frozen cells

1. Add culture medium (9 times the amount of stock solution) into the centrifuge tube for the preparation.

2. Transfer the thawed cell stock in the centrifuge tube while suspend them carefully using the culture medium in the centrifuge tube.

3. Centrifuge (room temperature, 400 G, 5 minutes) and then carefully remove the supernatant.

4. Add necessary amount of culture medium into the cell pellets and keep the cell density around 1.0 x 10^5 to 10^6 cells / mL, and count cell numbers.

5. Seed cells on the Corning® CellBind flask at a seeding density of 5000-7500 cells / cm^2.

6. Incubate in 37 ° C, 5% CO^2 incubator.

Caution: This is for reference only. Refer to the recommended methods of commercial cell preservation solution.