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, there 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.

Product Usage:

This medium is a non-clinical grade product and is for research use only. It should not be used for diagnosis or clinical applications!

Storage:

Please store in a dark place below -20 ° 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 2-8 ° 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.

NOTE! 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. Treat tissues such as adipose tissue with tissue dispersant such as collagenase to obtain a cell dispersed solution containing hMSC.
2. Add at least double the amount of this medium into cell dispersion, collect in a 50 mL centrifuge tube, and centrifuge (room temperature, 800G, 5 minutes or longer).
3. Carefully remove the supernatant, add 45 mL of the medium and resuspend well.
4. Centrifuge (room temperature, 400G, 5 minutes or longer) and carefully remove the supernatant.
5. Add 30 mL of the medium again and resuspend well.
6. After centrifugation (room temperature, 400G, 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).
(The recommended medium volume: 10 mL or more per 75 cm^2 of seeding area)
8. Incubate under 37°C, 5% CO2 conditions.
9. Observe the cell conditions daily 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.

NOTE! Since optimal culture conditions depend on different tissue types, this is for reference only. In addition, this medium does not contain antibiotics. Please add antibiotics as 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 volume of detached cell solution into a centrifuge tube.
4. Add an equal volume of this medium as the cell solution to the flask and wash the entire flask.
5. Add the entire volume to the centrifuge tube from step 3.
6. Centrifuge the cell detachment solution (room temperature, 400G, 5 minutes) and then carefully remove all supernatants.
7. Add more than 10 mL of this 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.
10. Add this medium to achieve a cell seeding density of 5000-10000 cells / cm^2.
11. Suspend cell solution well and seed cells on Corning® Cell BIND flasks.
12. Incubate under 37°C, 5% CO2 conditions.
13. Observe cell conditions and subculture when it reaches 70-90% confluence.
14. If the cell culture continues for more than 4 days, change the medium for every 3 days.

NOTE! 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 no dissociation agent remains.

This medium contains glutamine and glutamine alternatives. Antibiotics are not included, so please add as needed.

III. Seeding from frozen cells

1. Transfer the thawed cells to a centrifuge tube.
2. Wash the cell cryotube with this medium and collect the liquid in the centrifuge tube from step 1.
3. Add an appropriate amount of this medium to achieve a cell density of 1.0 x 10^5 - 10^6 cells/mL and perform cell counting.
4. Centrifuge (room temperature, 400G, 5 minutes) and then carefully remove the supernatant.
5. Add this medium to achieve a seeding density of 5000 - 10000 cells/cm2, and evenly seed in Corning® CellBIND® culture flasks.
6. Incubate under 37°C, 5% CO2 conditions.

NOTE! If you are using commercially available cell preservation solution, please prioritize the use of preserving solution.