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TheraPEAK™ MSCGM™ Mesenchymal Stem Cell Growth Medium

Instructions for use

Contents:

I. Introduction

TheraPEAK™ MSCGM™ Medium is a serum-free medium used for culturing human mesenchymal stem cells (hMSCs). TheraPEAK™ MSCGM™ Medium can be used to expand hMSCs. Cells can be directly transitioned from serum-containing medium to TheraPEAK™ MSCGM™ Medium with little to no adaptation time. Cells can be cultured in standard tissue culture treated flasks without the need for a coating matrix.

II. Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. Upon arrival, store TheraPEAK™ MSCBM™ Medium at 4°C to 8°C and the TheraPEAK™ MSCGM™ Supplement (2 x 5 mL vials) in the –20°C freezer (non-self-defrosting).
3. After both supplements are added to the basal medium, use within one month. Store at 4°C to 8°C in the dark. Do not refreeze.

III. Preparation of media

1. Thaw the TheraPEAK™ MSCGM™ Supplement vials (2 x 5 mL) at room temperature or in a 37°C water bath. Do not leave the supplement in the water bath longer than necessary.
2. In a biological safety cabinet, add the entire volume of the supplement (2 x 5 mL vials) to the bottle of TheraPEAK™ MSCGM™ Medium.
3. Rinse each of the supplement vials with 5 mL of medium and add to the basal medium. It may not be possible to recover the entire volume listed for the vials. Small losses should not affect media performance.
4. Store media at 2°C to 8°C in the dark.

NOTE: Smaller volumes of medium can be prepared, however the supplement vials should not be refrozen. Once thawed, the supplements can be stored at 2°C to 8°C for 1 week.

IV. Thawing of cells / initiation of culture process

1. The recommended seeding density for Human Mesenchymal Stem Cells is 5,000-6,000 cells per cm^2.
2. To set up cultures, calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. Add the appropriate amount of medium to the vessels (0.2 - 0.4 mL per $cm^{2)}$ and allow them to equilibrate in a 37°C, 5% CO_2 humidified incubator for at least 30 minutes. In addition to this, prepare a centrifuge tube with 5 mL of warmed media.
3. Wipe cryovial of cells with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, and then retighten. Quickly thaw the cryovial in a 37°C water bath.
4. Watch your cryovial closely; when the last sliver of ice melts, immediately remove it. Do not submerge it completely. Thawing the cells for longer then 1 ½ minutes results in less than optimal results.

5. Remove the cryovial immediately, wipe it dry, and transfer to a sterile field. Rinse the cryovial with 70% alcohol, and then wipe to remove excess.
6. Using a micropipette, gently add the thawed cell suspension to 5 mL of warmed media.
7. Centrifuge at 250-300 x g for 5 minutes at 2°C to 8°C.
8. Resuspend the pellet in a minimum volume of TheraPEAK™ MSCGM™ Medium by gently pipetting up and down. Count the total number of viable cells.
9. Add the calculated volume of cell suspension to each prepared flask and gently rock to disperse the cell suspension over the growth surface.
10. Incubate at 37°C, 5% CO_2 and 90% humidity.

V. Maintenance

1. hMSC cultures should be fed 3 - 4 days after plating.
2. To feed the cultures, gently and completely remove the TheraPEAK™ MSCGM™ Medium from the culture vessel.
3. Replace with an equal volume of warmed TheraPEAK™ MSCGM™ Medium and return the culture vessels to the incubator.

VI. Subculturing

1. Aseptically remove and discard all of the spent media from the flasks.
2. Wash the attached cell layer with HEPES-BSS (CC-5024) or an equivalent calcium and magnesium-free balanced salt solution. Add the wash solution to the side of the flask opposite the attached cell layer to prevent disruption. Rinse by rocking the flask back and forth several times. Aseptically remove and discard the wash solution.
3. Add a sufficient volume of trypsin-EDTA (CC-3232) or Animal-free cell dissociation reagent (TrypZean BE02-034E) to cover the cell layer (approx. 1 mL/25 cm^2). Gently rock the flask(s) to ensure that the cells are completely covered by the solution.
4. Incubate at room temperature for five minutes, and then observe under a microscope. If the cells are less than 90% detached, continue incubating and observe every 3 minutes. Tapping the flask or plate will expedite cell detachment. Do not incubate the cells longer than 15 minutes.
5. Once ≥90% of the cells are rounded and detached, stand the flasks on end for a minimal length of time to allow the cells to drain. Add 2 mL/25 cm^2 a soybean trypsin inhibitor (125 mg/L) to neutralize the trypsin. Wash flask by pipetting up/down several times to mix and collect into a sterile 15 mL or 50 mL conical tube.
6. Centrifuge cells at approximately 500 x g for five minutes at room temperature.
7. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated TheraPEAK™ MSCGM™ Medium and remove a sample for counting.
8. Count the cells with a hemacytometer or cell counter. If necessary, dilute the suspension with TheraPEAK™ MSCGM™ Medium to achieve the desired “cells/mL” and recount.
9. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}$$

10. Determine the total number of flasks to inoculate by using the following equation. The number of flasks needed depends upon cell yield and seeding density.

$$\text{Total \# of Flasks to innoculate} = \frac{\text{Total \# of viable cells}}{\text{Growth area} \times \text{Rec. Seeding Density}}$$

11. Use the following equation to calculate the volume of cell suspension to seed into your flasks. Determine the volume of TheraPEAK™ MSCGM™ Medium to add to each flask so that the final culture volume is 0.2 – 0.4 mL per cm^2.

$$\text{Seeding Volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 11}}$$

12. Prepare flasks by labeling each flask with the passage number, strain number, cell type and date.
13. Add the appropriate volume of temperature-equilibrated TheraPEAK™ MSCGM™ Medium.
14. Incubate at 37°C, 5% CO_2 and 90% humidity.
15. Three to four days after seeding, completely remove the medium. Replace with an equal volume of TheraPEAK™ MSCGM™ Medium. Cultures will be near confluence by day 6 or 7 and ready to subculture.

VII. Ordering information

BEBP18-936	TheraPEAK™ MSCGM™ Bulletkit™	TheraPEAK™ MSCBM™ Medium (BEBP12-934Q) **PLUS** TheraPEAK™ MSCGM™ Supplement (2 x BEBP13-935Z5)
BEBP12-934Q	TheraPEAK™ MSCBM™ Medium	Mesenchymal Stem Cell Basal Medium (1000 mL)
BEBP13-935Z5	TheraPEAK™ MSCGM™ Supplement	TheraPEAK™ MSCGM™ Supplement (2 x 5 mL)

VIII. Product use statement

TheraPEAK™ Media products are GMP produced and intended for further manufacturing use only. They are not intended for direct therapeutic use in humans.