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TheraPEAK™ Chemically Defined Mesenchymal Stem Cell Growth Medium (MSCGM-CD™)

Instructions for Use

Introduction

TheraPEAK™ MSCGM-CD™ is a serum free, chemically defined medium for the growth of human Mesenchymal Stem Cells. MSCGM-CD™ is optimized for the multiple passage expansion of all types of hMSCs while still allowing for differentiation into the several desired lineages. Cells can be directly transitioned from serum-containing medium to MSCGM-CD™ with little to no adaptation time.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. MSCGM-CD™ BulletKit™ Instructions: Upon arrival, store MSCBM-CD™ at 4℃ to 8℃ and the MSCGM-CD™ SingleQuots™ Kit at –20℃ in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4℃ and added to basal medium within 72 hours of receipt. After the one vial supplement of the SingleQuots™ Kit is added to basal medium, use within one month. Store at 4°C to 8℃ in a dark location. Do not refreeze.

Preparation of Media

1. Decontaminate the external surfaces of the MSCGM-CD™ SingleQuots Kit™ Cryovial and the Mesenchymal Stem Cell Basal Medium (MSCBM-CD™) bottle with 70% v/v ethanol or isopropanol.
2. Thaw the MSCGM-CD™ one vial supplement at room temperature. The supplement can also be thawed quickly in a 37℃ water bath. Do not leave the supplement in the water bath longer then necessary. Remove the supplement immediately once it has thawed completely.
3. Aseptically open the supplement. Using a pipette, add the entire volume from the vial to the bottle of MSCBM-CD™.
4. Rinse the cryovial with 5 ml of medium and add to the basal medium. It may not be possible to recover the entire volume listed for the cryovial. Small losses should not affect the cell characteristics.
5. Store at 2℃ to 8℃ in a dark location.

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

Coating Plates

For peak performance, a 5ug/mL fibronectin coating should be used. Let coating dry on surface overnight prior to seeding cells.

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 the vessels to equilibrate in a 37℃, 5% CO_2 humidified incubator for at least 30 minutes.

3. Wipe cryovial 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℃ water bath.
4. Watch your cryovial closely; when the last sliver of ice melts, 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 where the equilibrated flasks should be waiting, ready to seed. 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 temperature-equilibrated medium.
7. Centrifuge at 250-300 x g for 5 minutes at 2℃ t o 8℃.
8. Re-suspend the pellet in a minimum volume of temperature equilibrated Mesenchymal Stem Cell Growth Medium (MSCGM-CD™) 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℃, 5% CO_2 and 90% humidity.

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. Rinse by rocking the flask back and forth several times. Aseptically remove and discard the wash solution.
3. Add a sufficient volume either Trypsin-EDTA (CC-5012) or a NAO cell dissociation reagent to cover the cell layer (approx. 1 ml/25 cm^2). Gently rock the flask(s) to ensure that the cells are covered by the solution. 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.
4. 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 of a soybean trypsin inhibitor (125 mg/L) to neutralize the trypsin. Triturate cells several times to mix and pipette into a sterile 15 ml or 50 ml conical tube.
5. Centrifuge cells at approximately 500 x g for five minutes at room temperature.
6. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated MSCGM-CD™ and remove a sample for counting.
7. Count the cells with a hemacytometer or cell counter and calculate the total number of cells. Make a note of your cell yield for later use.
8. If necessary, dilute the suspension with MSCGM-CD™ to achieve the desired “cells/ml” and re-count the cells.
9. Assess cell viability using Trypan Blue.
10. Use the following equation to determine the total number of viable cells.

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

11. 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.

$$\text{Total \# of flasks to innoculate} = \frac{\text{Total \# of viablecells}}{\text{Growth area} \times \text{Rec. seeding density}}$$

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

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

13. Prepare flasks by labeling each flask with the passage number, strain number, cell type and date.

14. Add the appropriate volume of temperature equilibrated MSCGM-CD™ as determined in Step 12.
15. Incubate at 37℃, 5% CO_2 and 90% humidity.
16. Three to four days after seeding, completely remove the medium. Replace with an equal volume of MSCGM-CD™. Cultures will be near confluence by day 6 or 7 and ready to subculture.

Maintenance

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

Ordering Information

Cat. No.	Product	Description
00190632	MSCGM-CD™ Bulletkit™	500 ml MSCBM-CD™ plus 00192125 MSCGM-CD™ SingleQuot™ of growth supplement
00190620	MSCBM-CD™	Msenchymal Stem Cell Basal Medium (500 ml)
00192125	MSCGM-CD™ SingleQuots™ Kit	MSCGM-CD™ Growth Supplements, contains one vial

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH AND FURTHER MANUFACTURING USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.