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TheraPEAK™ Chemically Defined Dermal Fibroblast Cell Growth Medium (FGM-CD™)

Instructions for Use

Introduction

TheraPEAK™ FGM-CD™ is a serum-free, chemically defined medium for the growth of adult and neonatal human Dermal Fibroblast Cells. FGM-CD™ is optimized for multiple passage expansion of NHDFs. Cells can be directly transitioned from serum-containing medium to FGM-CD™ with little to no adaptation time. In addition, dermal fibroblasts grow on any culture ware and need no attachment matrix before adding FGM-CD™.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. FGM-CD™ BulletKit™ Instructions: Upon arrival, store FBM-CD at 4°C to 8℃ and the FGM-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. Thaw the one vial of FGM-CD™ supplement at room temperature. The supplement can also be thawed overnight at 4°C to 8℃ in a dark location.
2. Decontaminate the external surfaces of the FGM-CD™ SingleQuots Kit™ Vial and the Fibroblast Basal Medium (FBM-CD™) Bottle with 70% v/v ethanol or isopropanol.
3. Aseptically open the supplement. Using a 5ml or 10 ml pipette, add the entire volume from the vial to the bottle of FBM-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. Blend the supplemented medium either by pipetting with a large volume pipette or gently inverting the firmly closed bottle. Avoid shaking and frothing the medium.
6. We recommend that you place the supplied supplement label on the basal medium bottle once supplemented to avoid confusion.
7. Record the new expiration date on the label based on the shelf life of the reconstituted medium.
8. Store at 2°C 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°C to 8℃ for 1 week.

Thawing of Cells / Initiation of Culture Process

1. The recommended seeding density for adult human Dermal Fibroblast Cells is 2,000-3,500 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 the 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℃ 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 from the water bath and wipe dry. Spray the cryovial with 70% alcohol, and then wipe to remove excess.
6. Transfer the cryovial to a sterile field. Using a micropipette, gently add the thawed cell suspension to 0.5 ml of room temperature HEPES-BSS (CC-5024). Gently mix the cells by pipetting up and down. Count the total number of viable cells. Cells should not be centrifuged out of the cryoprotectant cocktail before addition to temperature-equilibrated culture flasks. This action is more damaging than the effects of DMSO residue in the culture.
7. Add the calculated volume of cell suspension to each prepared flask and gently rock to disperse the cell suspension over the growth surface.
8. Incubate at 37°C, 5% CO_2 and 90% humidity.
9. Thirty six to forty eight hours after seeding, completely remove the medium. Replace with an equal volume of FGM-CD™. Refeed cultures every two to three days. Subculture the cells at ≤ 80% confluence. Cultures reach 80% confluence between Day 5 and Day 6.

Subculturing

1. Passage the cells before reaching 80% confluence. Overly confluent cultures are difficult to dissociate and may require an alternate enzyme, such as Collagenase Type I.
2. Aseptically remove and discard all of the spent media from the flasks.
3. Wash the attached cell layer with HEPES- BSS 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. Allow the HEPES- BSS wash to remain on cells for two to three minutes. Aseptically remove and discard the wash solution.
4. Add a sufficient volume of either Trypsin-EDTA (CC-5012), an alternate dissociation enzyme 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 37°C 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.
6. For cultures dissociated with Trypsin/EDTA or Trypsin like NAO enzyme, 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.
7. For cultures dissociated with a non Trypsin enzyme, dilute cell suspension 1:10 with FGM-CD.
8. Centrifuge the harvested cells at 250 x g for five minutes to six minutes at room temperature. Aspirate the supernatant, except for 100-200µl.
9. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated FGM-CD™ and remove a sample for counting.
10. 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.
11. If necessary, dilute the suspension with FGM-CD™ to achieve the desired "cells/ml" and re-count the cells.
12. Assess cell viability using Trypan Blue or equivalent method.
13. Use the following equation to determine the total number of viable cells.

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

14. 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.

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

15. Use the following equation to calculate the volume of cell suspension to seed into your flasks.

$$\text{Seeding Volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 14}}$$

16. Prepare flasks by labeling each flask with the passage number, strain number, cell type and date.
17. Add appropriate volume of temperature equilibrated growth medium to new culture flasks.

18. After mixing the cell suspension uniformly, dispense the calculated volume into the prepared subculture vessels.
19. Incubate at 37°C, 5% CO_2 and 90% humidity.
20. Thirty six to forty eight hours after seeding, completely remove the medium. Replace with an equal volume of FGM-CD™. Refeed cultures every two to three days. Cultures will be near 80% confluence between 5 and 7 days and ready to subculture.

Maintenance

1. NHDF cultures should be fed 36-48 hours after plating and every two to three days, until subculture.
2. To feed the cultures, gently and completely remove the FGM-CD™ from the culture vessel.
3. Replace with an equal volume of temperature equilibrated FGM-CD™ and return the culture vessels to the incubator.

Ordering Information

00199041	FGM-CD™ Bulletkit™	FBM-CD™ (500 ml) plus SingleQuot™ (5ml) of growth supplement
00199019	FBM-CD™	Fibroblast Basal Medium
00199020	FGM-CD™ SingleQuot™ Kit	FGM-CD™ Growth Supplements, contains one vial

Related Products

CC-2511 NHDF-Ad Normal Human Dermal Fibroblasts-Adult

CC-2509 NHDF-Neo Normal Human Dermal Fibroblasts-Neonatal

12-769E Profreeze™-CDM Chemically Defined Freeze Medium

Product Use Statement

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