Recommended Expansion Protocol for Fed-Batch Culture Regimen with XF Bioreactor Starter Bundle

In order to seed and expand hMSCs (RoosterVial™-hBM/hUC-XF) using a cell culture feed regimen without a media exchange, you will need the following reagents, cell culture materials, and equipment:

1.0 MATERIALS& EQUIPMENT

ITEM	VENDOR*	PART No*	QUANTITY
XF Bioreactor Starter Bundle	RoosterBio	KT-056	1
RoosterNourish™-MSC-XF	RoosterBio	KT-016	1
Low Concentration Synthemax™ II Microcarriers	Corning	3781	6.25 g
DPBS (without Ca^{++}, Mg^{++})	Gibco	14190144	1
TrypLE™ Select Enzyme	Gibco	12563029	1
T225 CellBIND flasks	Corning	3293	1
10 mL costar wide tip serological pipettes	Corning	4492	1 package
100 µm cell strainer	BD Falcon	352360	1
150 mL sterile bottle	Corning	431175	1
500 mL centrifuge bottle	Corning	431123	1
Biosafety Cabinet			
Centrifuge			
Incubator			

* *Vendors and part numbers are included for critical items.*

2.0 MEDIA PREPARATION

2.1 Bring RoosterNourish-MSC-XF kit (SKU: KT-016) components to room temperature (RT) protected from light.

2.2 Prepare media by aseptically adding one bottle of RoosterBooster™-MSC-XF (Part No. SU-016) to one bottle of RoosterBasal™-MSC (Part No. SU-005/SU-022).

2.3 Mix well by capping and gently inverting the bottle.

3.0 CELL THAWING & SEEDING

3.1 Aseptically transfer 5 mL of prepared media into a 50 mL centrifuge tube.

3.2 Thaw RoosterVial-hBM/hUC-1M-XF (Part No. MSC-031/C43001UC) vial in an automated thawing device (e.g. ThawStar), or manually in a 37°C water bath. When thawing in a water bath, monitor the vial closely and remove from water bath once only a small bit of ice is remaining (2-3 min).

3.3 Aseptically transfer vial into a Biosafety Cabinet (BSC).

3.4 Transfer vial contents into the 50 mL centrifuge tube containing prepared media and mix cell suspension well.

3.5 Centrifuge at 300 x g for 5 min.
3.6 Aspirate the supernatant and resuspend cells in 45 mL of RoosterNourish™-MSC-XF media.
3.7 Mix well and seed cells into a T225 flask.
3.8 Transfer flask into an incubator (37°C, 5% CO_2) and ensure surfaces are covered with media.

4.0 CELL EXPANSION

4.1 Microscopically monitor cell confluency starting on day 3 of culture.
4.2 When culture is >80% confluent, cells are ready to harvest.

Day	Cell Confluency (%)
3	
4	
5	
6	
7	

5.0 CELL HARVEST & BIOREACTOR INOCULATION

5.1 In a BSC, weigh out 6.25 g of microcarriers into a sterile 150 mL bottle that allows for complete collection of liquid/microcarrier suspension.
5.2 Add 50 mL of growth media to the microcarriers and swirl to wet the microcarriers. Let the mixture sit for 10 minutes. This will wash the microcarriers and equilibrate them for culture.
5.3 Transfer the microcarrier suspension to a 500 mL PBS bioreactor. Use an additional 100 mL of growth media to rinse and transfer any remaining microcarriers from the 150 mL bottle.
5.4 Place the bioreactor in a 37°C incubator to equilibrate temperature/gas of the growth media until it is ready to be inoculated.
5.5 For harvesting the cells from the flask, transfer the vessel into a biosafety cabinet and remove spent media.
5.6 Add 10 mL of TrypLE to T225 flask.
5.7 Distribute TrypLE evenly to cover all the cells and place flask in 37°C incubator.
5.8 Check culture every 5 min until cells are detached from surface. Gently tap to dislodge remaining cells from surface.

Total Time Required for Cell Detachment

5.9 Add 10 mL of media to quench the TrypLE activity.

5.10 Transfer the cell suspension into a 50 mL centrifuge tube. Record volume of cell suspension.

Total Volume of Cell Suspension (A)

5.11 Mix well and transfer 0.5 mL of cells into a microcentrifuge tube for cell counts.
5.12 Count cells with a cell counting device, performing a dilution if required to get within its acceptable range.

Raw data		Adjusted data	
Dilution Factor (B)	Viable Cell Concentration (C)	Cell Concentration (D) = B * C	Total Viable cells at harvest (E) = D * A

5.13 Centrifuge cell suspension at 300 x g for 5 min.
5.14 Aspirate the supernatant and resuspend cells in fresh media to achieve a density of 1 M cells/mL.
5.15 Remove the bioreactor from the incubator and place in the BSC. Add 10.5 x 10^6 cells to the bioreactor that now contains the microcarriers and 100 mL of expansion media.
5.16 Swirl gently to mix and incubate at 37˚C for 20 min to allow cell attachment to microcarriers.
5.17 After 20 min, gently swirl the bioreactor containing the cells/microcarriers to redistribute the cells that have not adhered and the microcarriers to allow for contact to occur. Incubate at 37˚C for additional 20 min.
5.18 After 2 x 20 min incubation period, bring the bioreactor back into the BSC. Add additional 300 mL of growth media to bring the final volume to 450 mL.
5.19 Place the bioreactor on the magnetic base unit in 37°C incubator, and initiate agitation at 25 rpm.

6.0 BIOREACTOR FEEDING

6.1 On day 3 of culture, add 9-10 mL of RoosterReplenish™-MSC-XF (Part No. SU-023) (45-50x dilution) to the bioreactor, as a feed.
6.2 After adding RoosterReplenish-MSC-XF, the agitation rate should be increased up to 30 rpm to keep microcarriers in suspension.

7.0 BIOREACTOR SAMPLING

7.1 For bioreactor sampling, turn off the agitation and remove the bioreactor from the base unit in the incubator. Transfer both into a biosafety cabinet.

7.2 Begin agitating the bioreactor in the biosafety cabinet and let cells/microcarriers achieve a uniform suspension. This will be in the range of 30-35 rpm to achieve a uniform suspension.

7.3 Open the bioreactor cap and using a wide tip serological pipette aspirate 10 mL of uniform cell/microcarrier suspension into a 15 mL tube. (Additionally, 1 mL of cell/microcarrier suspension can be sampled to monitor cell attachment, growth, and aggregation via microscopy.)

7.4 For the 10 mL cell count sample, allow cells and microcarriers to settle into bottom of tube by gravity, typically 5-10 minutes depending on culture time.

7.5 Return the bioreactor/base unit to the incubator and continue agitation at 30 rpm.

7.6 Once the sample of cells and microcarriers settle, carefully remove as much spent media/ supernatant from the tube, without disturbing the cell/microcarrier suspension.

7.7 Add ~9 mL (equivalent to the volume of media removed from the sample; the volume of cells / microcarrier is typically 1 mL) of TrypLE solution to conical tube with cells/microcarriers and gently mix, then incubate for 15 min at 37˚C for cell dissociation from microcarriers. If large cell clumps remain, mix gently with pipette and return to 37˚C for an additional 10 mins.

7.8 When cells are dissociated from microcarriers, mix well and sample the cell solution to perform a cell count to determine cell density in bioreactor.

Day	Raw data		Adjusted data	
	Dilution Factor (B)	Viable Cell Concentration (C)	Cell Concentration (D) = B * C	Total Viable cells at harvest (E) = D * 450 mL
3				
4				
5				
6				
7				

Note that cells are typically ready to harvest on day 5-6 of culture and should be harvested during exponential growth phase (i.e., not when the cells have reach a growth plateau).

8.0 BIOREACTOR HARVESTING

8.1 For bioreactor harvesting, turn off the agitation and remove the bioreactor from the base unit for transfer into the biosafety cabinet.

8.2 Allow the cells/microcarriers to settle to the bottom of the bioreactor. Slightly push the wheel with a serological pipette to get the remaining cells/microcarriers from the wheel to settle to the bottom of bioreactor.

8.3 When all cells/microcarriers have settled, open the bioreactor cap and aspirate as much spent media from the culture as possible, without removing the cells/microcarriers.
8.4 Add 250 mL DPBS into the bioreactor and swirl gently to wash the cells/microcarriers.
8.5 Allow the cells/microcarriers to again settle to the bottom of the bioreactor, see step 8.2.
8.6 Aspirate the supernatant from the bioreactor, leaving cells/microcarriers.
8.7 Add 250 mL of TrypLE to bioreactor and transfer the bioreactor to 37°C incubator.
8.8 Incubate for 30 min with agitation at 100 rpm in the incubator. If large aggregates still exist, agitate for another 10 minutes in the incubator.
8.9 Transfer the bioreactor into the biological safety cabinet and measure the total volume of cells by transferring the cells/microcarrier solution into a sterile 500 mL centrifuge bottle.

Total Volume of Cell Suspension (X)

8.10 Pipette to break up remaining cell/microcarrier clusters.
8.11 When cells are dissociated from microcarriers, separate cells from the microcarriers using a 100 µm cell strainer.
8.12 Quench TrypLE with equivalent volume of fresh media.
8.13 Mix cell solution and obtain final cell count for the bioreactor harvest.

Raw data		Adjusted data	
Dilution Factor (B)	Viable Cell Concentration (C)	Cell Concentration (D) = B * C	Total Viable cells at harvest (E) = D * 2X