Recommended Expansion Protocol for RoosterVial-hBM-10M-XF

In order to expand one RoosterVial-hBM-10M-XF human bone marrow-derived Mesenchymal Stem/Stromal Cells (hBM-MSC) to at least 100 million (M) cells within one week, you will need the following reagents, cell culture materials, and equipment:

Expansion of RoosterBio XF hBM-MSC	
RoosterVial-hBM-10M-XF Lot No:	**Date/Time:**

1.0 MATERIALS & EQUIPMENT

ITEM	QUANTITY	VENDOR*	PART No*
RoosterVial-hBM-10M-XF	1 vial	RoosterBio	MSC-030
RoosterNourish™-MSC-XF	2 bottles	RoosterBio	KT-016
Either of the following: T225 CellBIND flasks 5-layer CellBIND CellSTACK (CS5)	 14 1	 Corning Corning	 3293 3311
TrypLE Select Enzyme	140 mL	Life Technologies	12563029
Biosafety Cabinet			
Centrifuge			
Incubator			

* Vendors and part numbers are included for critical items.

Please refer to the following Expansion Options table to determine the cell culture vessel best-suited to your research needs.

Expansion Options

Vessel	Surface Area of Vessel (cm^2)	Number Vessels Needed	Total Surface Area (cm^2)	Approximate Seeding Density (cells/cm^2)	Total Cells at Harvest	Days of Culture
T225	225	14	3,150	3,100	≥ 100M	3 to 7
CS5	3,180	1	3,180	3,100	≥ 100M	3 to 7

2.0 MEDIA PREPARATION

2.1 Bring RoosterNourish™-MSC-XF kit components to room temperature (RT) protected from light.

2.2 Prepare 2 bottles of 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 10 mL of prepared medium into a 50 mL centrifuge tube.

3.2 Thaw RoosterVial-hBM-10M-XF 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 medium and mix cell suspension well.

3.5 Centrifuge at 200 x g for 10 min.

3.6 Aspirate the supernatant and resuspend cells in 10 mL of RoosterNourish-MSC-XF medium. Transfer cells into 750 mL of RoosterNourish-MSC-XF.

3.7 Mix well and seed cells equally into fourteen T225 vessels or one CS5 vessel.

Type of culture vessel (X)	Total volume of cell suspension per vessel
T225 x 14 □	53 mL
CS5 x 1 □	750 mL

3.8 Transfer vessels 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

5.1 For harvest, transfer vessel(s) into biosafety cabinet and remove spent media.

5.2 Add 10 mL TrypLE to each T225 vessel or 125 mL TrypLE to each CS5 vessel.

5.3 Distribute TrypLE evenly to cover all the cells and place vessel(s) in 37°C incubator. 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

5.4 Add equivalent volume of RoosterNourish™-MSC-XF medium to each vessel to stop the TrypLE activity.
5.5 Transfer the cell suspension volumes into a 500 mL centrifuge tube.
5.6 Centrifuge at 500 x g for 10 min.
5.7 Aspirate the supernatant and resuspend cells in in medium to reach desired cell concentration. Measure the total volume of cell suspension.

Total volume of cell suspension (=A)

5.8 Mix well and transfer 0.5 mL of cells into microcentrifuge tubes for cell counts.
5.9 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 cells at harvest (E)=D*A

5.10 Cells are ready to be used in your application.

Note: One passage expansion is described above. If additional cells are required, second passage expansion options are listed below (select option based on required cell number at harvest). It is recommended that cells be used within 8-10 PDLs of the working cell bank.

Expansion Options —2nd Passage

Vessel	Surface Area of Vessel (cm^2)	Number Vessels Needed	Total Surface Area (cm^2)	Approximate Seeding Density (cells/cm^2)	Total Cells at Harvest	Days of Culture
CS10	6,360	5	31,800	3,000-5,000	≥ 750M	3 to 7
CS10	6,360	10	63,600	2,000-4,000	≥ 1.5B	3 to 7