Protocol for RoosterVial™-hBM-1M-XF EXPANSION

Expansion of RoosterBio XF hBM-MSC to yield 10 million cells	
RoosterVial-hBM-1M-XF Lot No:	Date/Time:

1.0 MATERIALS

ITEM	VENDOR	PART No*	EXP DATE
RoosterVial-hBM-1M-XF, (1 million (M) cells)	RoosterBio	MSC-031	
CTS TrypLE Select Enzyme	Life Technologies	A1285901	
DPBS (without Ca^{++}, Mg^{++})			
DPBS (without Ca^{++}, Mg^{++}) + hPL or SPENT MEDIA for quench			
hMSC High Performance Media Kit XF	RoosterBio	KT-016	
2 x T225 CELLBIND flasks OR 6 x T75 CELLBIND flasks	Corning		

* Vendors and part numbers are included for critical items.

2.0 MEDIA PREPARATION & CELL EXPANSION

2.1 Bring hMSC High Performance Media Kit XF to room temperature. Add 1 vial hMSC Media Booster XFM (SU-016) to 500 mL hMSC High Performance Basal Media (SU-005).

2.2 Obtain RoosterVial-hBM-1M-XF from liquid nitrogen dewar and immediately thaw in 37°C water bath. Monitor the process and remove from water bath once a small bit of ice is remaining (2-3 min).

2.3 Spray vial well with 70% isopropyl alcohol before transferring into biosafety cabinet.

2.4 Aseptically transfer cells into a 50 mL centrifuge tube.

2.5 Slowly (dropwise) add 4 mL of culture media to the cells.

2.6 Centrifuge at 200 x *g* for 10 min.

2.7 Carefully remove the supernatant without disturbing the cell pellet.

2.8 Resuspend the cells in 5 mL of culture media. When cells are resuspended bring volume up to 30 mL with culture media.

2.9 Mix well and seed cells equally into two T225 or into six T75 vessels, and add media to bring volume up to final volume:

Type of culture vessel (X)	Total volume of cell suspension per vessel	Cells/ cm^2	Final Volume/ flask
T75 x 6 □		2,222	15ml
T225 x 2 □		2,222	45ml

2.10 Transfer vessel(s) into 37°C incubator and ensure that the surfaces are covered with media.
2.11 Microscopically observe culture everyday from day 3 onwards to determine percentage confluency.
2.12 If culture is less than 50% confluent on day 3, perform a media change. Completely remove the spent media from the vessel, and replace with same volume of fresh culture media. Transfer vessel back into incubator.
2.13 When culture is >80% confluent, prepare to harvest the following day.

Day	Confluency (%)
3	
4	
5	
6	
7	

3.0 CELL HARVESTING

3.1 For harvesting, transfer vessel into biosafety cabinet and remove spent media.
*Collect ~10 mL spent media in sterile container if using to quench harvest enzyme.
3.2 Remove media and add 10 mL of TrypLE to T225 or 3 mL TrypLE to each T75 flask, and incubate in 37°C incubator.
3.3 Check culture every 5 min until cells are detached from surface. Gently tap to dislodge remaining cells from surface.

Total time required for cells detachment

3.4 Add equal volume of quench or spent media to stop the TrypLE activity.
3.5 Transfer the cell suspension into a 50 mL centrifuge tube.

Total volume of cell suspension (=A)

3.6 Centrifuge at 200 x *g* for 10 min.
3.7 Aspirate the supernatant and resuspend cells with 4-5 mL of fresh media.
3.8 Measure the total volume of cell suspension.
3.9 Mix well and transfer 0.1 mL of cells into microcentrifuge tubes for cell counts.
3.10 Dilute cells to 0.5 mL with /DPBS to get counts in the range of 0.1-1 x 10^6 cells/mL.
3.11 Mix well and cells are ready for counts with cell counting device.

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

3.12 Place harvested cell suspension in 4°C refrigerator for use within 1 hour of harvest.