# Recommended Expansion Protocol for RoosterVial-hBM-1M-XF

In order to expand one vial of RoosterVial-hBM-1M-XF human bone marrow-derived Mesenchymal Stem/Stromal Cells (hBM-MSC) to at least 10 million cells within one week, you will need the following reagents, cell culture materials, and equipment:

| **Expansion of RoosterBio XF hBM-MSC** | |
|---|---|
| **RoosterVial-hBM-1M-XF Lot No:** | **Date/Time:** |

## 1.0 MATERIALS & EQUIPMENT

| ITEM | QUANTITY | VENDOR* | PART No* |
|---|---|---|---|
| RoosterVial-hBM-1M-XF | 1 vial | RoosterBio | MSC-031 |
| RoosterNourish™-MSC-XF | 1 bottle | RoosterBio | KT-016 |
| Either of the following:<br>T75 CellBIND flasks<br>T225 CellBIND flasks | <br>4<br>2 | <br>Corning<br>Corning | <br>3290<br>3293 |
| TrypLE Select Enzyme | 20 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. Note: If larger cell numbers are required, a decrease in seeding density to 2,200 cells/cm$^2$ (and increase in total vessel seeding surface area to 450 cm$^2$ and total seeding media to 90 mL) will maximize total cell output at harvest with an increase in Population Doubling Level (PDL).

### 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 |
|---|---|---|---|---|---|---|
| T75 | 75 | 4 | 300 | 3,333 | ≥ 10M | 3 to 7 |
| T225 | 225 | 2 | 450 | 2,222 | ≥ 12M | 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 1 bottle of medium by aseptically adding 1 bottle of RoosterBooster™-MSC-XF (Part No. SU-016) to 1 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-1M-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 30 mL of RoosterNourish-MSC medium.
3.7 Mix well and seed cells equally into four T75 vessels or into two T225 vessels, and add medium to bring volume up to final volume according to table below:

| Type of culture vessel (X) | Total volume of cell suspension per vessel | Final volume per vessel |
|---|---|---|
| **T75 x 4** □ | 7.5 mL | 15 mL |
| **T225 x 2** □ | 15 mL | 45 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 vessels into biosafety cabinet and remove spent media.
5.2 Add 3 mL TrypLE to each T75 flask or 10 mL TrypLE to each T225 flask.

5.3 Distribute TrypLE evenly to cover all the cells and place vessels 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 to each vessel to stop the TrypLE activity.
5.5 Transfer the cell suspension into a 50 mL centrifuge tube.
5.6 Centrifuge at 200 x g for 10 min.
5.7 Aspirate the supernatant and resuspend cells 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 | 1 | 6,360 | 3,000-5,000 | ≥ 75M | 3 to 7 |
| CS10 | 6,360 | 2 | 12,720 | 2,000-4,000 | ≥ 150 M | 3 to 7 |

*Caution to Users: RoosterBio products contain human sourced materials and should be treated as potentially infectious. Employ universal safety precautions and wear protective clothing and eyewear while handling. Practice appropriate disposal techniques per CDC guidelines for biohazardous material.*