Recommended Transduction Process for RoosterGEM™

Protocol Summary

RoosterGEM is a complete chemically defined medium that has been engineered for increased efficiency and integration of lentiviral vectors into mesenchymal stromal cells (hMSCs) and other primary cell types, without the need for additional supplementation.

While RoosterGEM functions as a stand-alone medium, it has been optimized for use with RoosterBio hMSCs expanded using RoosterNourish™-MSC-XF (part no. K82016, protocols are available at www.roosterbio.com). The general process recommendations are outlined below.

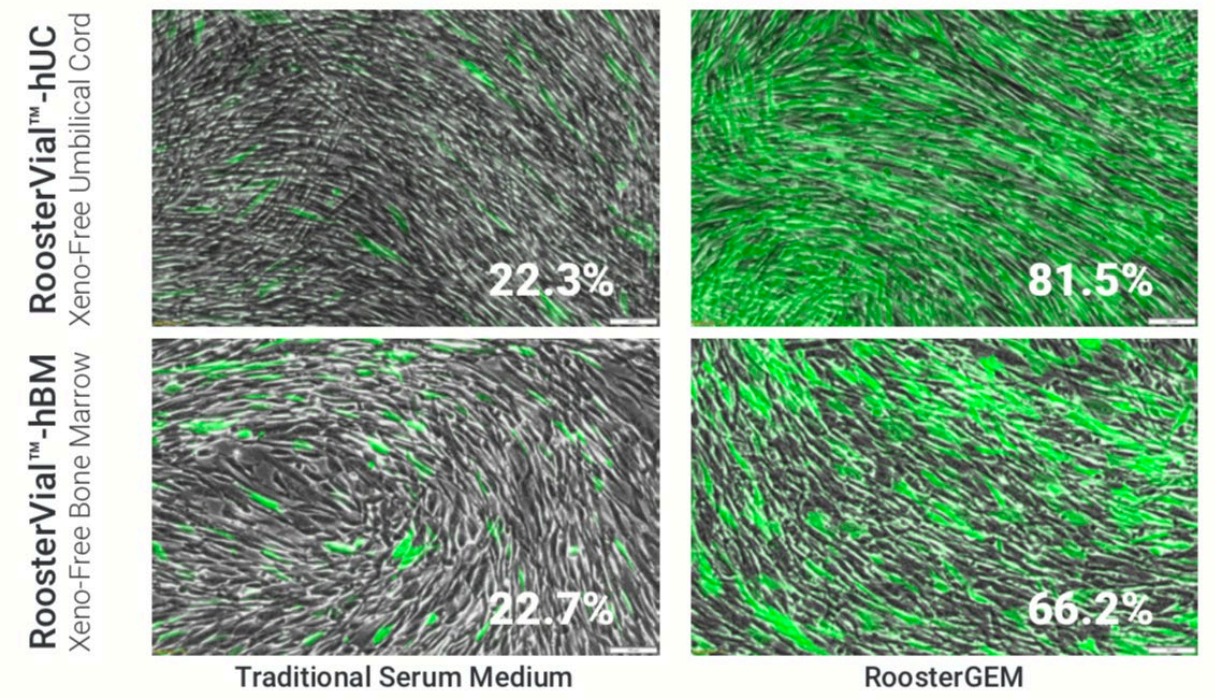


Figure: RoosterGEM Increases Transduction in hMSCs Derived from Multiple Tissue Sources

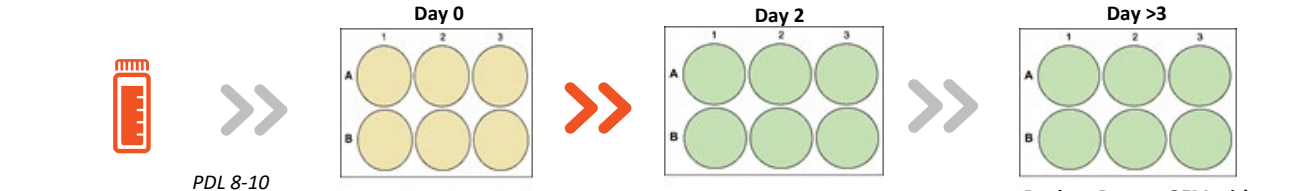
Increased transduction efficiencies were achieved in RoosterBio's Xeno-free (XF) RoosterVial™ hMSCs from bone marrow- and umbilical cord-derived tissue sources (part no. MSC-031 and C43001UC). Lentiviral transduction was performed at MOI of 2 with rLV.EF1.ZsGreen1-9 (Flash Therapeutics).

Lentiviral Transduction of hMSCs in 2D culture surface

Note: The optimal MOI for each lentiviral vector lot must be empirically determined. A screening experiment titrating from MOI 2-50 is strongly recommended.

Process Overview

Cell Expansion Summary



**RoosterBio strongly recommends the use of Corning CellBIND, Vitronectin, or Fibronectin surfaces for expansion of the Xeno-Free product line.*

- Thaw and seed cells at recommended: 3,000-5,000 cells/cm^2 in RoosterNourish-MSC-XF and allow for proliferation for up to ~48 hours.
- Incubate cultures for ~24 hours in RoosterGEM + lentiviral vector (MOI from 2 to 50; 1 ml RoosterGEM per 9.5 cm^2).
- Replace with 2 ml per well RoosterNourish-MSC-XF. Do not rinse wells.
- Monitor for integration and expression of transgene (~48-72 hours).

Recommended Protocol

1. Shipping and Storage

1.1. RoosterGEM is shipped frozen, and, upon receipt, promptly, store RoosterGEM at -20°C.
1.2. Before use, thaw RoosterGEM at ~2-8°C away from light until no ice remains (typically 24-48 hours).
1.3. After use, return RoosterGEM to ~2-8°C away from light for up to 2 months from initial thaw.

2. Cell Culture

2.1. For 6-well CellBIND plates, seed 3,000 to 5,000 hMSCs/cm^2 (30k to 50k hMSCs per well) in expansion medium (RoosterNourish-MSC-XF, part no. K82016).
2.1.1. Note: It is recommended that 6-well plates be utilized for optimization of expression. Once optimal conditions are determined, process can be proportionally scaled accordingly (1 ml RoosterGEM per 9.5 cm^2).
2.2. If desired, plate additional well to count cells per well prior to genetic modification step.
2.3. Allow cultures to proliferate for 2 days.

3. Media Preparation

3.1. Aliquot the required volume of RoosterGEM (allow for 1.2 ml per well of 6-well plate) into a separate conical tube and warm to room temperature, protected from light.
3.2. Promptly return unused RoosterGEM bottle to 2-4°C storage away from light.
3.2.1. Store for use up to 2 months from thaw.

4. Genetic Modification

Note: For initial screening experiments, an MOI from 2 to 50 is strongly recommended.
4.1. Count cells from a single well with a counting device.
4.1.1. Aspirate RoosterNourish-MSC-XF from a single well.

4.1.2. Add 750 ul TrypLE to one well of 6 well plate.
4.1.3. Distribute TrypLE evenly to cover all the cells and place vessels in 37°C incubator.
4.1.4. Check culture every 5 min until cells are detached from surface.
4.1.5. Add equivalent volume of RoosterNourish™-MSC-XF to each vessel to stop the TrypLE activity.
4.1.6. Transfer the cell suspension into an appropriate centrifuge tube.

4.2. Count cells with a cell counting device, performing a dilution if required to get within its acceptable range.

Cells (per well)

4.3. Calculate the amount of lentivirus needed per N reactions (6-well plate):

Cell Count (per 6-well, 9.5 cm^2) **(=A)**	**Volume of RoosterGEM** (1ml per 9.5 cm^2) **(V=1ml*N)**	**Desired MOI (=B)**	**Volume of Lentivirus per 1mL RoosterGEM D=A*B/virus titer(TU/mL)**

4.4. Formulate the RoosterGEM w/ lentiviral vector as calculated above.
4.5. Aspirate RoosterNourish-MSC-XF from the remaining wells.
4.6. Replace with 1 ml per well RoosterGEM containing lentiviral vector and incubate for 24 hours.
4.7. Aspirate RoosterGEM + lentiviral vector and replace with 2 ml per well RoosterNourish-MSC-XF. Do not rinse wells.
4.8. Monitor for integration and expression of transgene [Typical time is 2 to 3 days]

5. Cell Harvest

5.1. Transfer vessel(s) into biosafety cabinet and remove spent medium.
5.2. Add 1mL TrypLE to each well of 6 well plate.
5.3. Distribute TrypLE evenly to cover all the cells and place vessels in 37°C incubator.
5.4. Check culture every 5 min until cells are detached from surface. Gently tap to dislodge remaining cells from surface.
5.5. Add equivalent volume of RoosterNourish™-MSC-XF to each vessel to stop the TrypLE activity.
5.6. Transfer the cell suspension into an appropriate centrifuge tube.
5.7. Centrifuge at 250 x g for 10 min.
5.8. Aspirate the supernatant and resuspend cells in medium to reach desired cell concentration. Measure the total volume of cell suspension.
5.9. Mix well and transfer 0.5 mL of cells into microcentrifuge tubes for cell counts.
5.10. Count cells with a cell counting device, performing a dilution if required to get within its
5.11. acceptable range.
5.12. Cells are ready to be used in your application.

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