

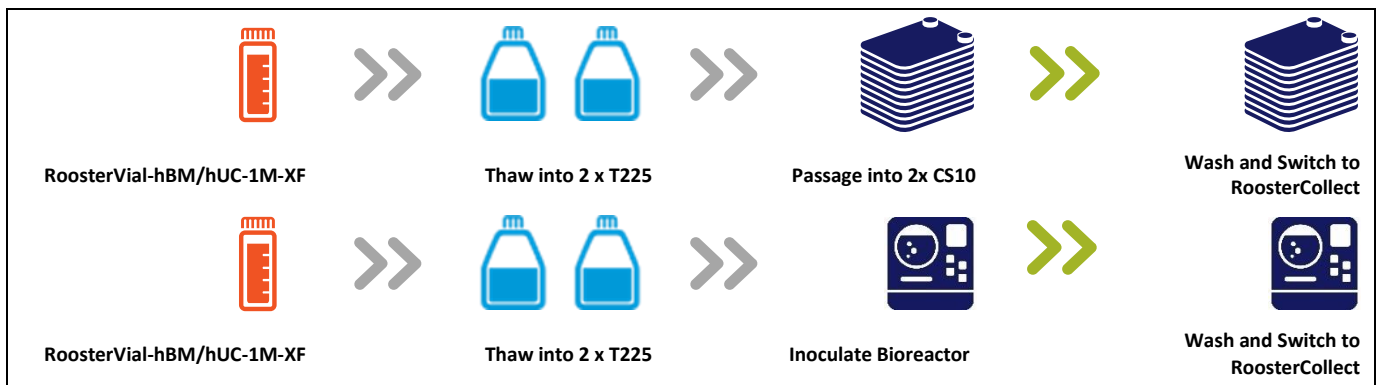
Recommended EV Collection Protocol with RoosterCollect-EV

Protocol Summary

RoosterCollect-EV (M2001) is a chemically defined, protein-free bioprocess medium used to collect hMSC Extracellular Vesicles (EVs) from hMSCs expanded in either 2D flask culture or 3D bioreactor culture platforms. Please refer to the section below that is specific to your culture system:

Process Overview

Protocol Summary



**RoosterBio strongly recommends the use of Corning CellBIND surfaces for expansion of the Xeno-Free product line.
**Please refer to protocol for full process instructions.*

Recommended 2D Flask Protocol

1. Cell Culture

- 1.1. Culture cells according to RoosterBio hMSC Expansion Protocols provided with RoosterBio hMSC systems or custom protocol.
- 1.2. When culture is >80% confluent (typically after >4-5 days in culture), proceed with the following steps.

2. Media Preparation

- 2.1. Allow RoosterCollect-EV to warm to room temperature away from light for up to 4 hours.

3. EV Collection and Harvest

- 3.1. Transfer cell culture vessels, room temperature RoosterCollect-EV or equivalent wash solution, and other necessary materials to biosafety cabinet.
- 3.2. Wash cultures to removed impurities and residuals from RoosterNourish.
 - 3.2.1. Aspirate spent medium from cell culture flasks.
 - 3.2.2. Add equivalent working volume of RoosterCollect-EV, or equivalent wash solution.
 - 3.2.3. Aspirate the volume added in step **3.2.2**.

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M2001 RoosterCollect™-EV

- 3.2.4. For more thorough removal of impurities and residuals from the growth medium, repeat steps **3.2.1-3.2.3** with an added wash incubation (37°C, 5% CO₂) for 15 minutes. If not needed, proceed to step **3.3**.
- 3.3. Add equivalent working volume of RoosterCollect-EV.
- 3.4. Return flasks to incubator (37°C, 5% CO₂) for up to 48 hours.
- 3.5. After culture time, harvest conditioned media for particle collection.

Recommended Bioreactor Protocol

4. Cell Culture

- 4.1. Culture cells according to RoosterBio hMSC Bioreactor Expansion Protocols or custom protocol.
- 4.2. When cultures reach desired cell density (cells/mL) typically by day 5, proceed with the following steps.

5. Media Preparation

- 5.1. Allow RoosterCollect-EV to warm to room temperature away from light for up to 4 hours.

6. EV Collection and Harvest

- 6.1. Transfer cell culture vessels, room temperature RoosterCollect-EV, and other necessary materials to biosafety cabinet.
- 6.2. Allow cells/microcarriers to settle to the bottom of the bioreactor.
- 6.3. Wash cultures to removed impurities and residuals from RoosterNourish.
 - 6.3.1. Open the bioreactor cap and aspirate as much spent medium from the culture as possible, without removing the cells/microcarriers.
 - 6.3.2. Add half the working volume of RoosterCollect-EV, or equivalent wash solution, (e.g. 200-250 mL for a 0.5L bioreactor) to the bioreactor and swirl to wet the microcarriers.
 - 6.3.3. Allow cells/microcarriers to settle to the bottom of the bioreactor.
 - 6.3.4. Aspirate as much wash medium from the culture as possible, without removing the cells/microcarriers.
 - 6.3.5. Repeat steps **6.3.1-6.3.4** for a second wash. If not needed, proceed to step **6.4**.
- 6.4. Add complete working volume of RoosterCollect-EV.
- 6.5. Return bioreactor to incubation (37°C, 5% CO₂) and standard agitation for up to 72 hours.
 - 6.5.1. Agitation may be slightly increased if aggregation is observed (+5 rpm every 24 hours).
- 6.6. After culture time, allow cells/microcarriers to settle and harvest conditioned media for particle collection.

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.

Provision of Seller Product subject to Seller Standard Terms and Conditions. Any technical advice furnished, or recommendation made concerning any use or application of any Seller Product is believed to be reliable, but Seller makes no warranty, either express or implied, as to its accuracy or completeness or of the results to be obtained. Purchaser assumes full responsibility for quality control, testing and determination of suitability of Seller Product for its intended application or use.

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