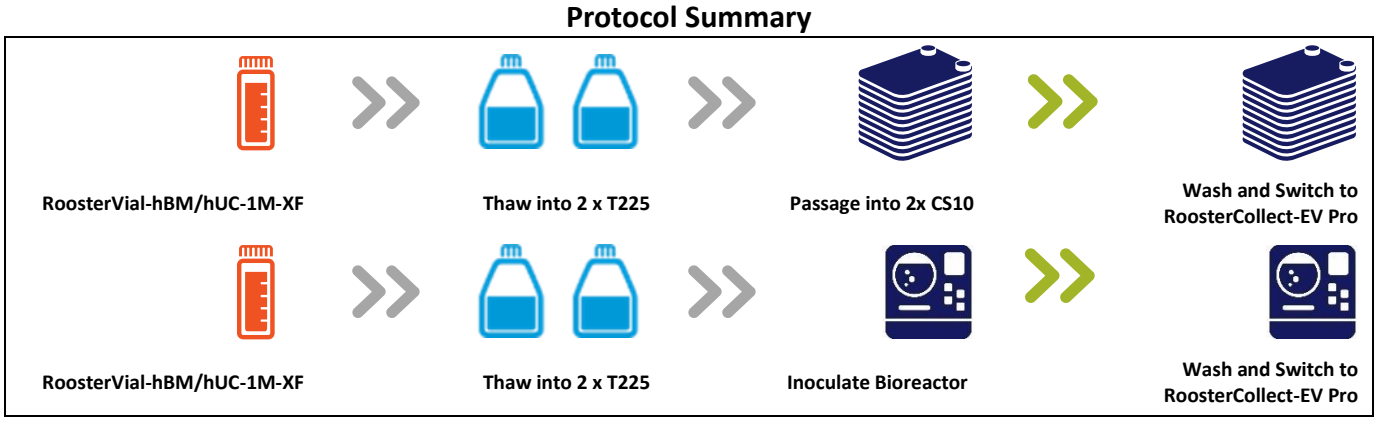
Recommended EV Collection Protocol with RoosterCollect-EV Pro

Protocol Summary

RoosterCollect-EV Pro (K41001) is comprised of RoosterCollect™-EV, a low-particle medium, and EV Pro™, a supplement meant to be used with RoosterCollect-EV, to increase EV yield and support cell health for extended EV collection. This chemically defined bioprocess medium can be 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



**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 >60-80% confluent (typically after >3 days in culture), proceed with the following steps.

2. Media Preparation

2.1. Allow RoosterCollect-EV (M2001) and EV Pro (S44002) to warm to room temperature away from light for up to 4 hours.
2.2. Prepare RoosterCollect EV Pro by adding EV Pro to RoosterCollect-EV at a 1:50 dilution.
2.2.1. Transfer media and necessary materials to biosafety cabinet. Aseptically add EV Pro (10 mL) to RoosterCollect-EV medium (500 mL) using a pipette to prepare RoosterCollect EV Pro.
2.2.2. Mix well by gently pipetting the solution up and down until evenly mixed.

3. EV Collection and Harvest

3.1. Transfer cell culture vessels, room temperature RoosterCollect-EV Pro, RoosterCollect-EV base medium or equivalent wash solution, and other necessary materials to biosafety cabinet.
3.2. Wash cultures to remove 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**.
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% CO_2) for 15 minutes. If not needed, proceed to step **3.3**.
3.3. Add equivalent working volume of RoosterCollect-EV Pro.
3.4. Return flasks to incubator (37°C, 5% CO_2) for 4-6 days.
3.5. After culture time, harvest conditioned media for particle collection and downstream processing/purification.

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 day 4-6 days, proceed with the following steps.

5. Media Preparation

5.1. Allow RoosterCollect-EV (M2001) and EV Pro (S44002) to warm to room temperature away from light for up to 4 hours.
5.2. Transfer media and necessary materials to biosafety cabinet.
5.3. Prepare RoosterCollect-EV Pro by adding EV Pro to RoosterCollect.
5.3.1. Recommended final working concentration of EV Pro should equal 2%.
5.3.2. While calculating dilution, for instance adding 10mL of EV Pro to 500mL working volume of RoosterCollect-EV, the dead volume (volume remaining within the bioreactor after Step **6.3.5**) should be compensated for.

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 remove 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 appropriate working volume of RoosterCollect-EV Pro to the bioreactor.
6.5. Return bioreactor to incubation (37°C, 5% CO_2) and standard agitation for 4 to 6 days.
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 and downstream processing/purification.