

## **Recommended EV Collection Protocol with RoosterCollect™-EV**

RoosterCollect-EV (M2001) is a 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:

### **2D Flask hMSC EV Collection**

#### **1.0 CELL CULTURE**

- 1.1 Culture cells according to RoosterBio hMSC Expansion Protocol provided with RoosterBio hMSC systems or custom protocol.
- 1.2 When culture is >80% confluent, proceed with the following steps.

#### **2.0 MEDIA PREPARATION**

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

#### **3.0 EV COLLECTION AND HARVEST**

- 3.1 Transfer cell culture vessels, room temperature RoosterCollect-EV, and other necessary materials to biosafety cabinet.
- 3.2 Aspirate spent medium from cell culture flasks.
- 3.3 Add replacement volume of RoosterCollect-EV and return flasks to incubator (37°C, 5% CO<sub>2</sub>) for 48 hours.
- 3.4 After culture time, harvest conditioned media for particle collection.

## **Bioreactor hMSC EV Collection**

### **1.0 CELL CULTURE**

- 1.1 Culture cells according to RoosterBio hMSC Bioreactor Expansion Protocol or custom protocol.
- 1.2 After 5-6 days of culture, following RoosterBio hMSC Bioreactor protocol, or desired cell density with custom protocol, proceed with the following steps.

### **2.0 MEDIA PREPARATION**

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

### **3.0 EV COLLECTION AND HARVEST**

- 3.1 Transfer bioreactor, DPBS, room temperature RoosterCollect-EV, and other necessary materials to biosafety cabinet.
- 3.2 Allow cells/microcarriers to settle to the bottom of the bioreactor.
- 3.3 When all cells/microcarriers have settled, open the bioreactor cap and aspirate as much spent medium from the culture as possible, without removing the cells/microcarriers.
- 3.4 Add half the working volume of DPBS (ex. 250mL DPBS for a 0.5L bioreactor) to the bioreactor and swirl to wet the microcarriers.
- 3.5 Allow cells/microcarriers to settle to the bottom of the bioreactor.
- 3.6 When all cells/microcarriers have settled, open the bioreactor and aspirate as much DPBS from the culture as possible, without removing the cells/microcarriers.
- 3.7 Add the replacement volume of the removed media of RoosterCollect-EV (ex. 500mL for a 0.5L bioreactor) and return the bioreactor to the incubator (37°C, 5% CO<sub>2</sub>) for 3 days.
- 3.8 After culture time, allow cells/microcarriers to settle and harvest conditioned media for particle collection.