

Process Recommendations for EV Collection with RoosterCollect[™]-EV and EV Boost[™]

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. EV Boost (S2001) is an optional, tunable reagent to be used with RoosterCollect-EV to increase collected particles over shortened culture durations.

1.0 MEDIA PREPARATION

- 1.1 Allow Rooster Collect-EV and EV Boost to warm to room temperature away from light for up to 4 hours.
- 1.2 Prepare Rooster Collect-EV + EV Boost according to desired output for application. See table below for formulating Low and High Boost recommendations, based on testing with the RoosterBio hMSC and RoosterNourish[™]-MSC system.
 - 1.2.1 Transfer media and necessary materials to biosafety cabinet. Aseptically add EV Boost to Rooster Collect-EV medium using a pipette to prepare Rooster Collect-EV+ EV Boost.
 - 1.2.2 Mix well.

Table 1: Please refer to this table for recommended High and Low EV Boost concentrations tested with RoosterBio XF-hMSCs expanded in RoosterNourish-MSC-XF. The effect of these concentrations on total particles collected is shown in Figure 1 below.

| RoosterCollect-EV + EV Boost | | | |
|-------------------------------------|----------------------|--------------------------------------|-------------------------------------|
| EV Boost Dose | EV Boost (mL) | RoosterCollect-EV Medium (mL) | Suggested Culture Time (hrs) |
| Low | 10 | 500 | 24 |
| High | 30 | 500 | 6 |

2.0 EV COLLECTION AND HARVEST

- 2.1 Transfer cell culture vessels to biosafety cabinet.
- 2.2 Aspirate supernatant from cell culture vessels.
- 2.3 Add replacement volume of prepared RoosterCollect-EV + EV Boost and return to incubator (37°C, 5% CO₂) for suggested culture time according to Table 1 or specific to custom application.
- 2.4 After desired culture time, harvest conditioned media for particle collection.

Process Note: EV Boost is a tunable research reagent that should be optimized for your application. High and Low EV Boost concentrations are recommended starting points based on particle collection using RoosterBio hMSCs as the cell source.

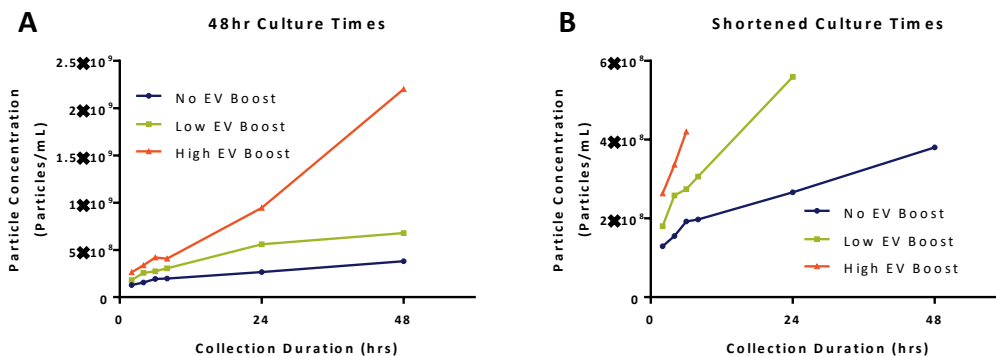


Figure 1: Particle concentration generated from RoosterBio XF hBM-MSCs at varying concentrations of EV Boost-supplemented RoosterCollect-EV. **(A)** Increased particle yield with Low and High EV Boost concentrations. **(B)** Shortened collection time for similar particle yield with increased EV Boost concentrations. **Note:** Extended use of High EV Boost can induce cell death that accompanies the increased particle yield.