

Maximizing MSC-EV Yield with Preserved Quality Using a Xeno-Free and Defined Formulation

Clemence Bonhomme¹, Karthi Rajamani², Loora Laan², Diwas Srivastava², Ilona Szucs¹, Cottrell Tamessar³, Jane Synnergren³, Catharina Brandsten¹, Kathryn Murray¹, Tarik Hadi²

1 Takara Bio Europe Swedish Filial, Göteborg, Sweden
2 Takara Bio Europe, Saint-Germain-en-Laye, France
3 Systems Biology Research Center, University of Skövde, Sweden

Abstract

Human mesenchymal stem cells (hMSCs) have strong therapeutic potential due to their self-renewal and multi-lineage differentiation abilities. In recent years, MSC-derived extracellular vesicles (EVs) have emerged as a promising cell-free therapeutic approach, retaining the regenerative and immunomodulatory properties of MSCs. However, scalable EV production without compromising quality remains a major challenge. By screening a bank of molecules known to enhance EV release, we developed a formulation that increased EV yield by up to nine-fold compared to DMEM. Importantly, in line with MISEV guidelines, EV size, morphology (TEM), and surface markers CD63, CD9 and CD81 remained consistent across conditions. Transcriptomic analysis further confirmed that RNA cargo remained stable, closely mirroring primary MSC signatures.

Together, these findings demonstrate that our EV production media, Cellartis® MSC EV Wonder™, in combination with Cellartis® MSC XF medium for expansion of MSCs, enables high-yield, high-quality EV production; offering a robust, scalable solution for both research and future clinical applications. The MSC EV Wonder is a complete medium free from animal-derived components. It is specifically formulated to increase the production of EVs from MSCs. It allows a direct shift from MSC expansion to EV collection without adaptation, simplifying workflows and improving efficiency.

- **Defined formulation** – Ensures consistent results
- **Phenol Red-Free** – Ideal for downstream EV analysis
- **Seamless Expansion-to-Production** – No adaptation needed
- **High EV Yield** – Optimized to maximize EV output
- **Multi-Source MSC Support** – Validated for BM-, AD-, and UC-derived MSCs
- **Low Particle Background** – Cleaner isolations with reduced interference

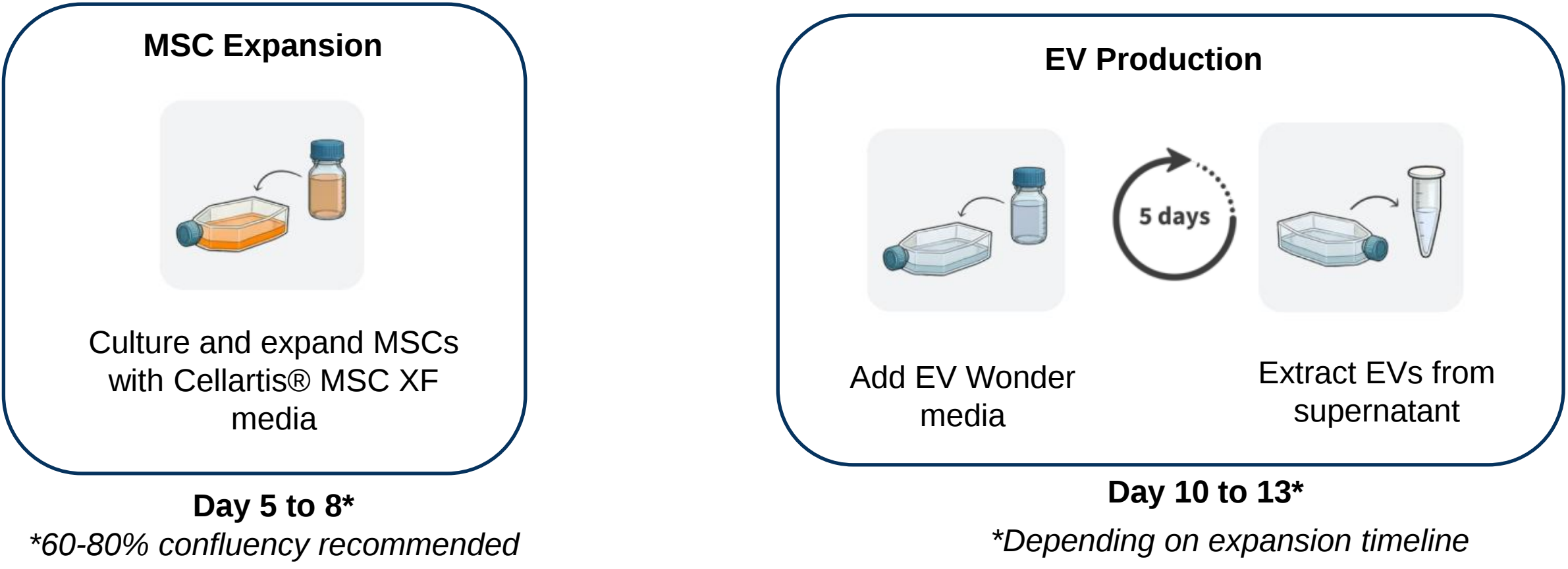


Figure 1. Streamlined process from MSC expansion to EV production. To minimize variability and streamline EV workflow, we used a simple two-phase process using Cellartis® MSC Xeno-Free Culture Medium (Cat# Y50200/ Cat#Y50205) for MSC expansion and Cellartis® MSC EV Wonder™ (Y51000) for EV production. The complete process from expansion to production required 10 to 13 days depending on the initial seeding density.

2 Screening strategy for identification of compounds enhancing EV production

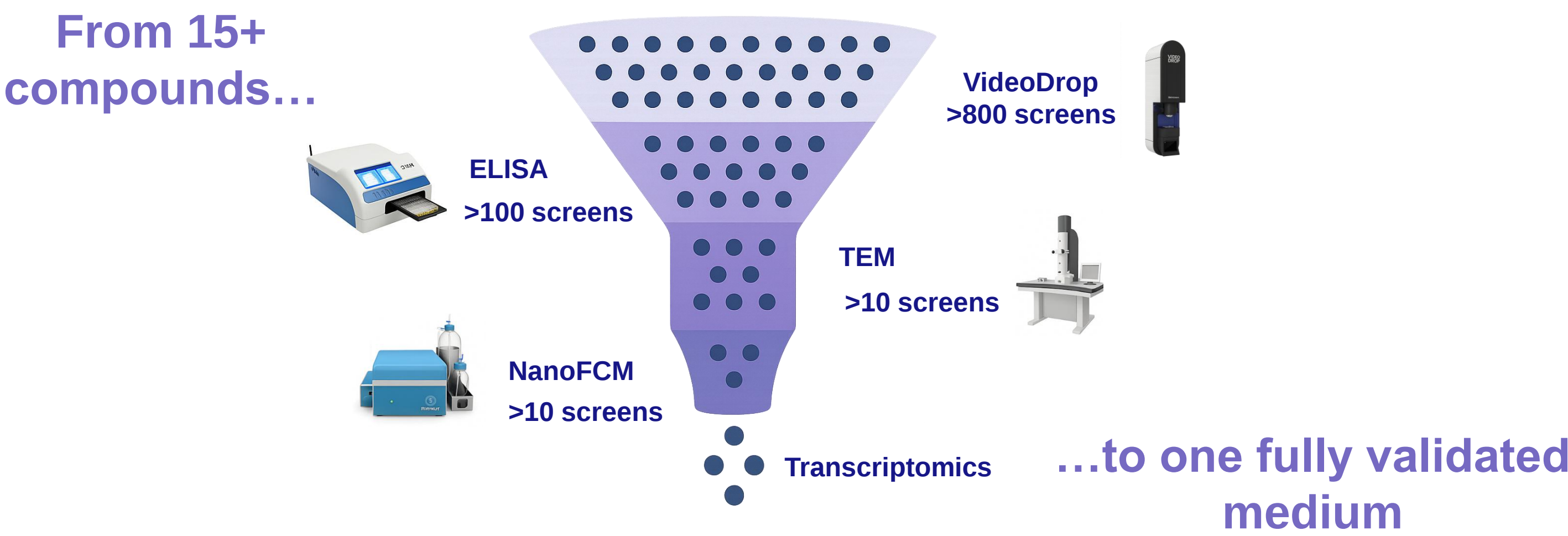


Figure 2. Screening strategy for identification of compounds enhancing EV production. During the development of MSC EV Wonder, more than 15 candidate compounds were evaluated using sequential assays including VideoDrop (>800 screens), ELISA (>100 screens), NanoFCM (>10 screens), and TEM (>10 screens). Transcriptomic analysis of selected candidates led to the identification of one fully validated medium formulation.

3 EV production using MSlevels up to 9-fold higher compared to DMEM

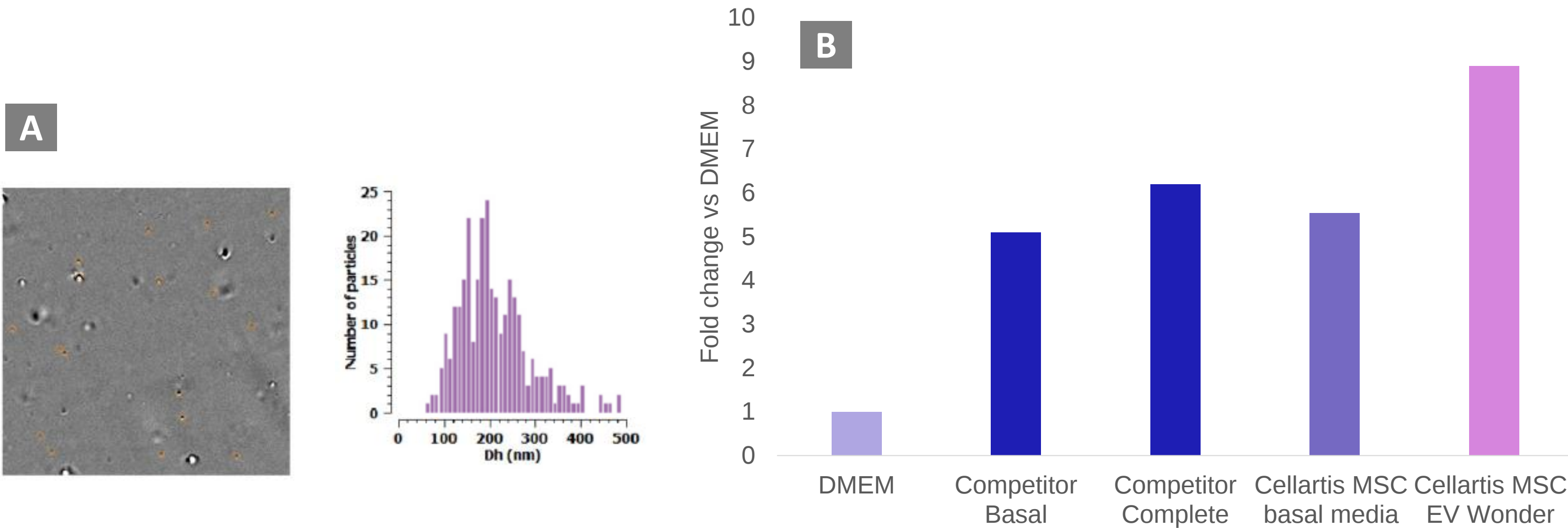


Figure 3. High particle number observed using VideoDrop analysis of MSC derived EVs. MSCs were cultured in MSC Xeno-Free Culture Medium, followed by EV production using MSC EV Wonder for 5 days. (A) Particles/ml were quantified using VideoDrop. Representative images of visualizaition and quantification by VideoDrop. (B) Using MSC EV Wonder, a 9-fold increase in particle concentration was observed compared to DMEM.

4 EV quantity and quality superior to commercial competitor media

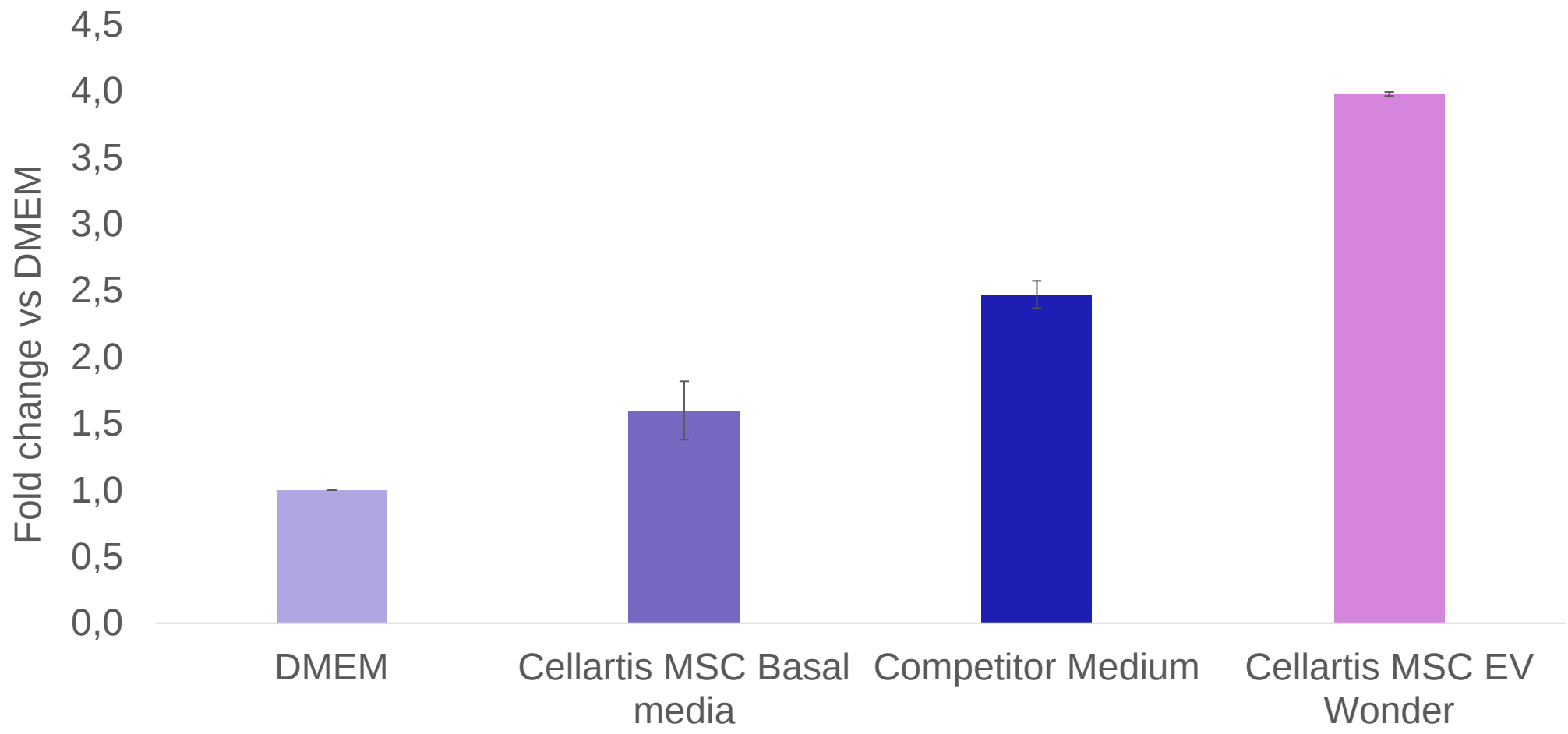


Figure 4. Quality of EVs maintained along with high yield. EVs produced using Cellartis® MSC EV Wonder™ show significantly higher number of CD63 positive particles, a key exosomal marker associated with vesicle identity and functional potential. As measured by ELISA, MSC EV Wonder resulted in increased CD63 signal compared to a leading commercial medium, confirming superior exosome quality and marker expression.

5 High purity of EVs without comprising EV quantity

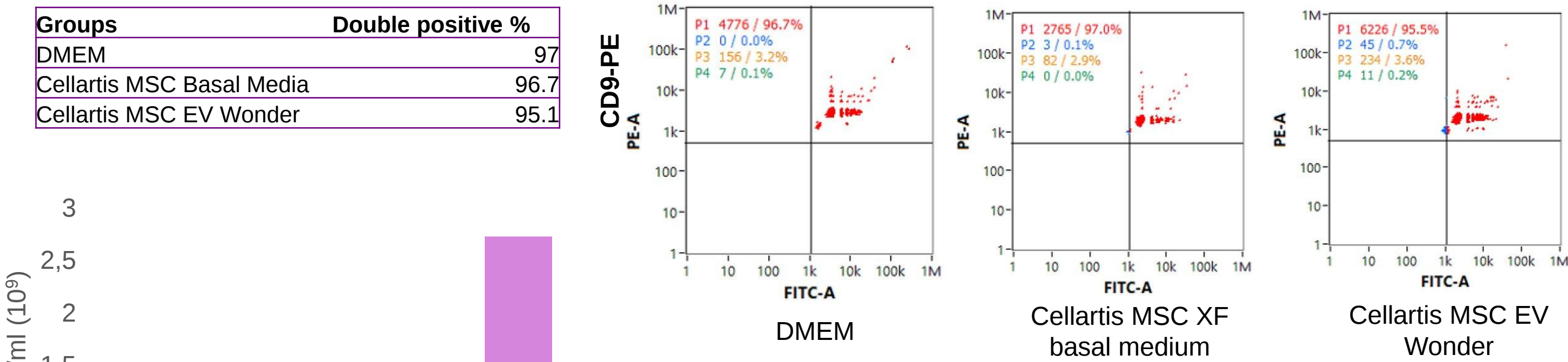


Figure 5. MSC EV Wonder allows for production of high quantity of EVs without compromising EV quality. EVs produced using Cellartis MSC EV Wonder demonstrated significantly high CD9+/CD81+ double-positivity confirming high EV purity across conditions. Importantly, MSC EV Wonder supported substantially higher EV yield while maintaining this high purity profile.

6 EVs developed have consistent size and morphology

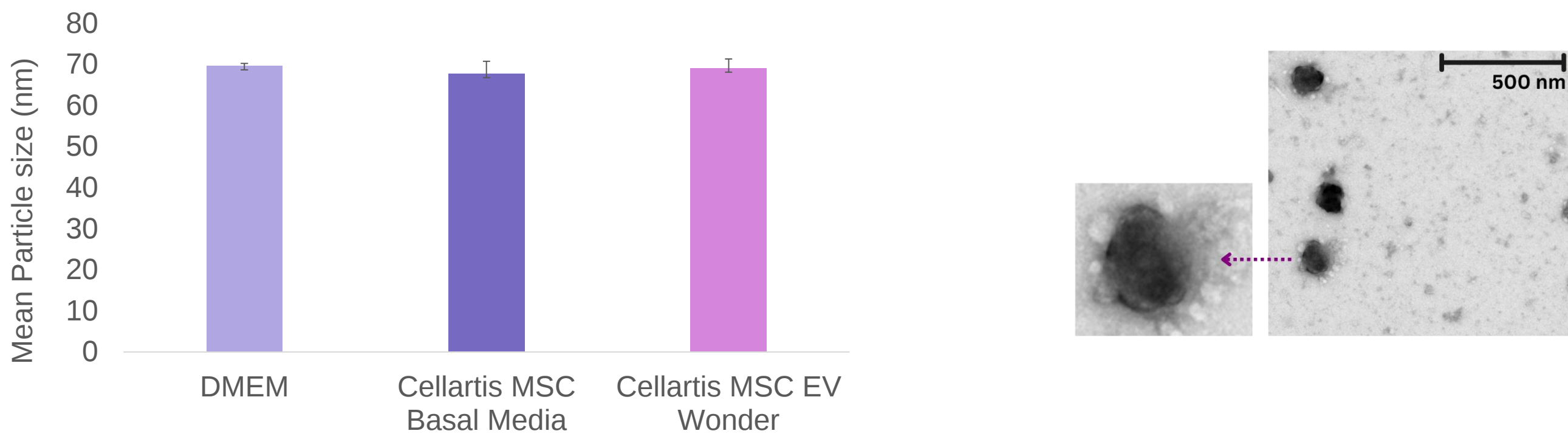


Figure 6. Consistent EV size with characteristic morphology. Using the MSC EV Wonder protocol, EVs were produced with a mean particle size of ~69 nm, consistent with the expected size of extracellular vesicles. This uniformity across media highlights the reliability of the protocol for generating high-quality MSC-derived EVs. The TEM image (with inset zoom) illustrates the typical round morphology of vesicles; scale bar = 500 nm.

7 Compatible across multiple sources of MSCs

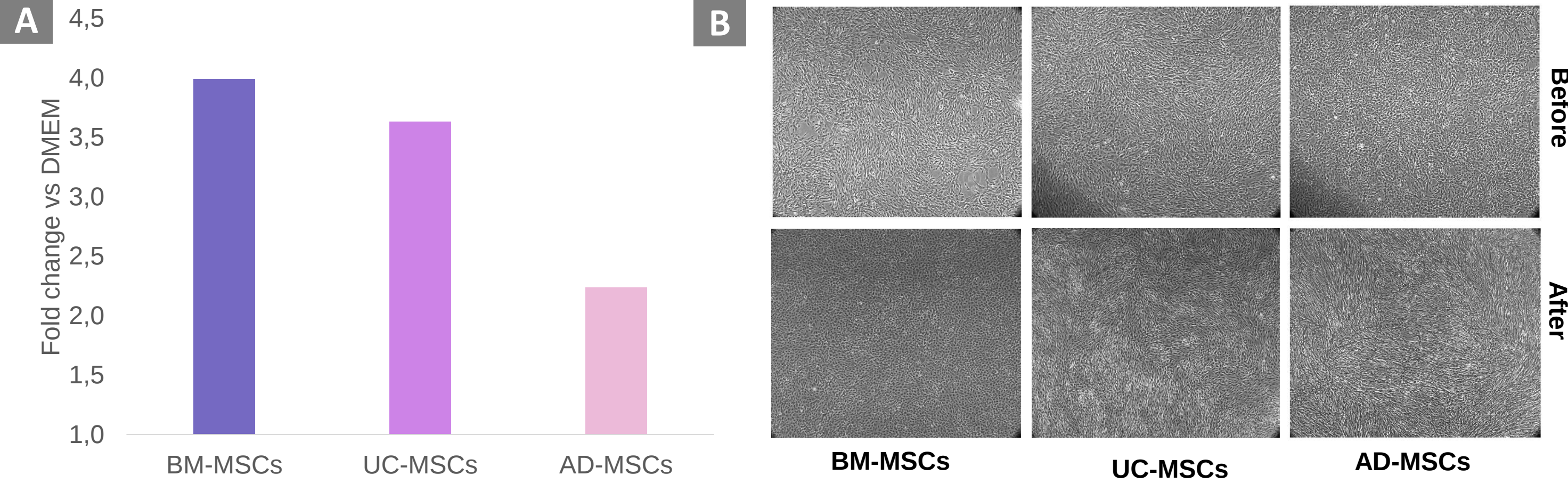


Figure 7. EV Wonder enhances EV yield across BM-, UC-, and AD-MSCs. Cells were expanded in Cellartis MSC media, and EV production was performed with MSC EV Wonder for 5 days. (A) ELISA analysis of CD63 expression showed increased EV yield compared to DMEM across all MSC sources tested. Representative images of BM-MSCs, UC-MSCs, and AD-MSCs before (B) and after (C) treatment with MSC EV Wonder are shown.

Conclusions

- Cellartis MSC EV Wonder is complete media that can be used to produce high quantity of EVs while maintaining the quality of Evs.
- The media also works with multiple MSC sources (BM-MSCs, AD-MSCs, and UC-MSCs)
- The media portfolio from Cellartis allows streamlined expansion to production under xeno-free conditions.
- Through various methodologies, below mentioned EV characteristics were confirmed
 - CD marker expression profiles
 - Correct size and morphology



Scan the QR code to get your test bottle today! Limited time only!

