

# To the Standardization of Exosome Isolation and Characterization



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## ABSTRACT

Research involving exosomes is rapidly expanding with a vast increase in the quality and quantity of publications. An improved and more efficient isolation protocol for exosomes is critical to advancing this exciting field. Challenges to researchers working with exosomes include setting up density gradients by hand, because it is tedious, time-consuming and subject to user, lab, and method variability. There also is a need for greater accuracy in size and concentration analysis. At the same time, experts in the field have called for the establishment of standard protocols. This poster focuses on solutions to those challenges through cost-effective, large-scale purification, and fast analysis of exosomes. Specifically, the Biomek 4000 Workstation helps to overcome human variables and provides a consistent, reproducible, high-throughput method for gradient setup and fractionation. Optima Ultracentrifugation Series helps researchers maintain reliability between runs, making outcomes highly reproducible. The DelsaMax CORE saves time and cost of TEM analysis for size and concentration.

## INTRODUCTION

Exosomes are small vesicles, most often described in the literature to be less than 100 nm and have been demonstrated to be released by a wide variety of cell types, exhibiting a multitude of functions, and proven to be involved in cancer metastasis. Exosome purification and analyses comprise a fast, evolving research area; more than 70% of published research on exosomes has been done within the last six years. Standardization of isolation and characterization methods is critical to the advancement of this exciting, emerging field. Ultracentrifugation is frequently the preferred choice for exosome isolation, generating highly pure sample preparations; however, the workflow is lengthy and often lacks reproducibility among laboratories and users. The most tedious step involves layering and fractionating from a density gradient and often contributes the most disparity.

Here we present a simple workflow using automation, centrifugation and dynamic light scattering (DLS), to purify and analyze exosome samples. The Biomek 4000 Laboratory Automation Workstation helps overcome the human variable and provides a consistent, reproducible, high-throughput method for density gradient layering and fractionation—an elegant solution to scale-up hurdles. Preparative ultracentrifugation helps to maintain reliability between runs and offers high reproducibility, achieving timely separation of biological macromolecules without marker-based biases. The DelsaMax CORE DLS platform is used for size analysis of the density-gradient fractions. The system allows exosomes to be: (1) analyzed in free-solution, (2) with statistical significance, and (3) with less cost and time, compared to Electron Microscopy. Instead of taking several hours to analyze a few hundred particles, the DelsaMax CORE is able to analyze and size thousands of exosomes in one minute. The outlined workflow can be simply modified to other applications and exosome sources and represents the importance of using automated, standardized methods for exosome research and reporting.

## METHOD

### CELL CULTURE

Exosomes are derived from many sources including nearly all bodily fluids, cell types, and species; however, cell culture remains a popular approach for the study of extracellular vesicles. Beckman Coulter's Vi-Cell is an automated viability counter and offers cell-type differentiators for optimized cell counts. Researchers can normalize for expression against cell number and the Vi-Cell makes the process quick, easy, and non-biased.



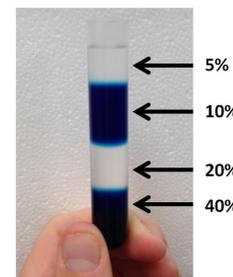
## GRADIENT LAYERING & FRACTIONATION

Centrifugation is a preferred method for exosome isolation as the technique presents no capture biases. The centrifugation protocol involves more than 5 differential steps, but the result is highly pure particles of proper size and shape. An additional tedious, yet very critical step, in isolation is the isopycnic density gradient. Here, four different densities of gradient solutions (iodixanol) are layered in succession with sample on top and allowed to sediment to a solution density matching that of the sample. The tube is then fractionated and exosomes are recovered for downstream analysis.



To reduce the difficult and often non-reproducible protocol, Beckman Coulter's Biomek 4000 Workstation was employed to automate the process. Increasing densities of iodixanol were underlaid in a centrifuge tube and sample was loaded on top. The tube was spun in an SW-41 rotor and fractionated by liquid level tracking from the top of the tube using the Biomek 4000. The process generated a tight inter-face between density layers, and fractions were easily recovered in a timely fashion.

Gradient Layer	Density (g/mL)	Volume (mL)	% Iodixanol (0.25M Sucrose; pH 7.5)
1	1.160	3	40
2	1.147	3	20
3	1.133	3	10
4	1.120	2	5



## CENTRIFUGATION

Seven centrifugal steps are required for highly pure exosome samples; Beckman Coulter, a world leader in the centrifugation business for over 70 years, offers a centrifuge and rotor for each individual step. The schematic below represents the entire workflow utilizing 3 different Beckman Coulter centrifuges.



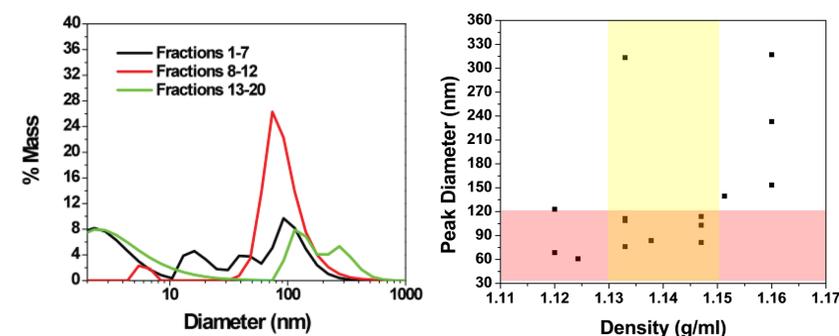
- Allegra X-15R
  - 750 x g, 10 min, 25°C
  - SX4750A rotor
  - Beckman 50 mL conical tubes
- Allegra X-15R
  - 2,000 x g, 20 min, 4°C
  - SX4750A rotor
  - Beckman 50 mL conical tubes
- Optima XPN
  - 10,000 x g, 20 min, 4°C
  - SW 32 Ti rotor
  - Polypropylene Bell-Top Quick-Seal, 15 mL
- Optima XPN
  - 100,000 x g, 40 min, 4°C
  - SW 32 Ti rotor
  - Polypropylene Bell-Top Quick-Seal, 15 mL
- Optima XPN
  - 100,000 x g, 18 hours, 4°C
  - SW 41
  - Ultra-Clear, 13.2 mL, 14 x 89 mm
- Optima MAX-XP
  - 100,000 x g, 45 min, 4°C
  - TLA 120.2 rotor
  - Quick-Seal, 1.5 mL, 11 x 25 mm
- Optima MAX-XP
  - 100,000 x g, 45 min, 4°C
  - TLA 120.2 rotor
  - Quick-Seal, 1.5 mL, 11 x 25 mm

## RESULTS & DISCUSSION

### CHARACTERIZATION

Exosome characterization can take on several forms. Researchers are typically initially interested in size information to confirm the purity of their purified product. Electron microscopy and dynamic light scattering are the two most popular techniques, but EM can be costly and take considerable amounts of time. The follow-up characterization approach involves probing the contents of the purified product by Western blotting for protein receptors and/or deep sequencing of the encapsulated RNA population. Here, we illustrate the DelsaMax CORE DLS instrument which is a cost-effective alternative to electron microscopy and offers unparalleled precision in particle characterization in the nanometer scale.

The gradient was fractionated and these fractions were combined for particle analysis, representing the top of the gradient (black), middle (red), and bottom (green). The middle fractions demonstrated a size profile centered around 100 nm in diameter and a density between 1.12 g/mL – 1.16 g/mL as expected for exosomes.



## CONCLUSIONS

Exosomes are becoming a major research area in science as both diagnostic and therapeutic biomarkers. Additionally, exosomes have been shown to be part of intercellular communication networks and implied in tumor regulation. However, the field is still in its infancy, and often times, the size range and exosome RNA content are misreported or vary from publication to publication. In order to advance this field, it is essential to standardize the workflow to purify and better characterize the entire exosome population for a more representative sample as a tool in the fight against cancer development and metastasis.

## FUTURE OUTLOOK

Size profiling is only one component of exosome characterization. Beckman Coulter's future intentions are to automate the entire workflow for RNA extraction and next-generation sequencing using the Biomek platform.

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