CELL guidance systems

Application Note

Comparison between Precipitation & Size Exclusion Chromatography (SEC) vs. Membrane Affinity for exosome isolation

Exosome isolation from cell culture conditioned medium using Exo-spin™ Midi Columns and Membrane Affinity

Data Courtesy of Dr. Triantafillos Liloglou and Dr. Amelia Acha-Sagredo
University of Liverpool, United Kingdom (Issued March, 18th 2019)

- **Experiment**
  Isolate exosomes using Exo-spin™
- **Exosomes origin**
  Conditioned cell culture medium
- **Initial sample volume**
  10 ml per Exo-spin™ Midi Column
- **Elution sample volume**
  500 µl per fraction in PBS

Summary

In an effort to reduce lung cancer mortality rates through understanding mechanisms and improved early diagnosis, much research focus has recently been oriented towards extracellular vesicles (EVs). This is both in the context of EV biogenesis and also biomarker potential.

The research reported here compared two exosome isolation methods applied to conditioned cell culture medium from lung cancer cells. Results from samples pre-treated with Exo-spin™ Buffer and purified using Exo-spin™ Midi Columns were compared to the purification efficacy of a membrane affinity spin column method.

The Exo-spin™ purification system has been identified as superior to the membrane affinity technique. This was described through two major benefits: 1) exosomes do not aggregate; and 2) exosomes are ready to use without further buffer exchange, even in functional downstream applications. Analyses such as particle number and vesicle size have been performed.

Methods

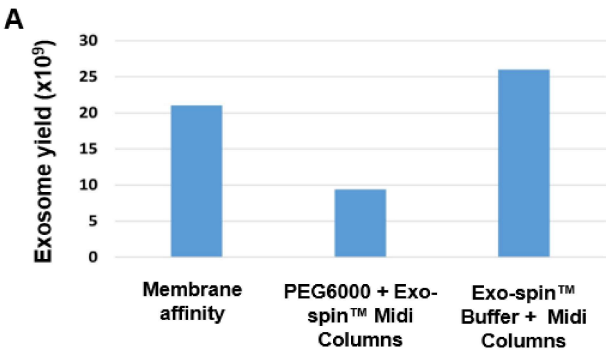
Exo-spin™ Midi Columns

1. Remove cells and cellular debris
2. Precipitate exosomes containing pellet using Exo-spin™ Buffer (optional)
3. Purify exosomes using Exo-spin™ Midi SEC Columns

Membrane affinity

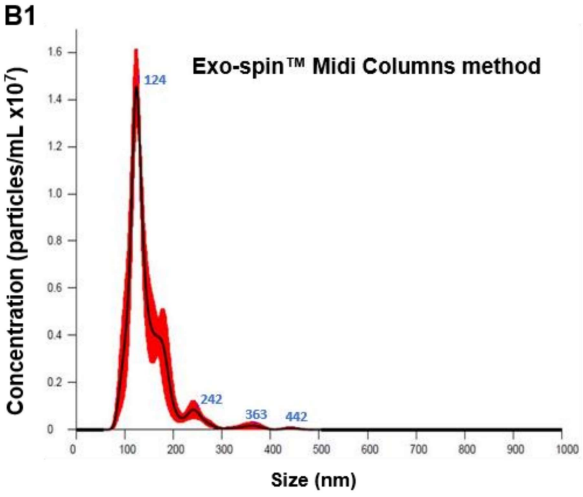
1. Remove cells and cellular debris
2. Mix samples with binding buffer and bind to column
3. Remove unbound particles by washing
4. Elute EVs in elution buffer

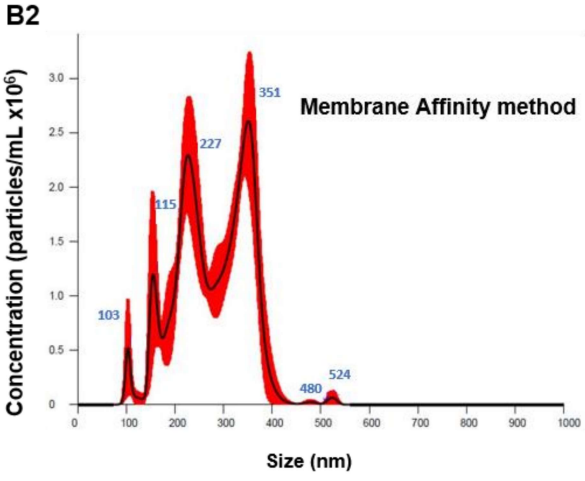
Results



(A) Comparison of total yields from 10 ml conditioned cell culture medium by EV isolation method. Total particle yield obtained with Exo-spin™ Buffer + Exo-spin™ Midi Columns is greater than the yield obtained using the membrane affinity method. Notably, Exo-spin™ Buffer is much more efficient than generic PEG 6000 when used prior to Exo-spin™ Midi Column exosome isolation.

(B) NTA of EV fractions. EV samples were isolated from 10 ml conditioned medium. **(1)** The overwhelming majority of particles isolated with the Exo-spin™ technology were in the 100-150 nm range, following a narrow size distribution expected for highly pure, high quality exosomes. **(2)** Vesicles isolated using the membrane affinity method, showed a broad range of particles with the majority in the 200-400 nm region. Blue numbers indicate peak particle sizes.





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