ISEV 2023
(Poster PF10.02)

LipoMin™ - Removal of Lipoproteins from SEC- or UC-Treated Samples towards High-Quality Isolation of EV within 12 Min.

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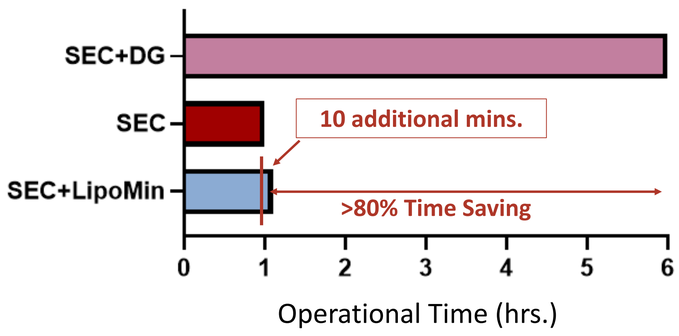
Introduction

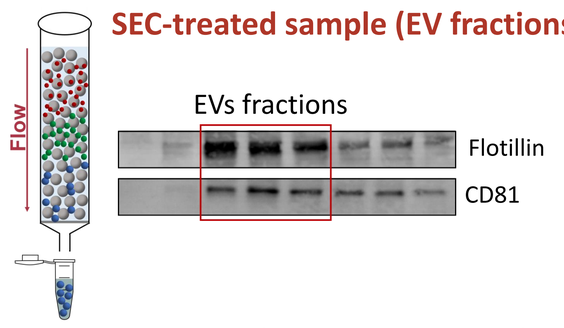
- Extracellular vesicles (EV) isolation from plasma or serum is complicated by the abundance of lipoprotein (LP), which has similar size and density to EVs, leading to low-purity EV isolation. Nevertheless, significant advancement has been made in sequential processing based on size and density of particles, such as size exclusion chromatography (SEC) followed by density gradient (DG). The two-step process removes considerable LP contamination from EV-fractions in post-SEC exosomal fraction(s).
- However, DG requires ultracentrifugation (UC), making the process time-consuming. Further, stand-alone UC process (without SEC) co-isolates undesirable high-density-LP (HDL) in the EV pellet. Commercial LP removal reagents, mostly based on LP antibodies, have been used with some success.
- **LipoMin™** reagent contains functionalized magnetic beads to remove LP (both ApoA & ApoB) from SEC-, UC-, or DG-treated samples, achieving high-purity EV within 12 minutes. (See below for SEC + LipoMin, as an example.)

SEC + LipoMin™ Saves Time (>80%)



Methods

SEC-treated sample (EV fractions)

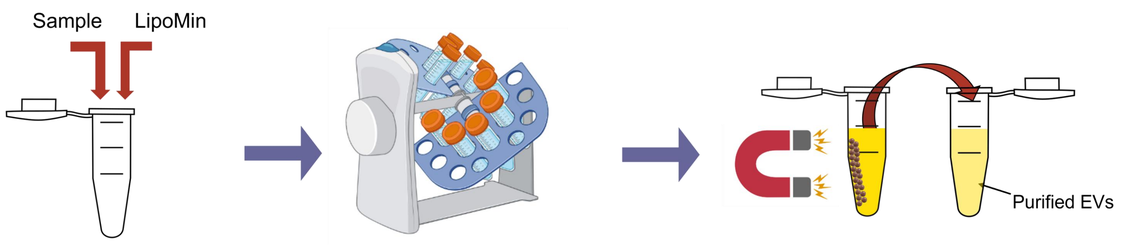


UC-treated sample

Ultracentrifugation at 100,000 × g for 2 hrs. × 2 times

The LipoMin protocol requires a simple incubation & mixing steps after reagent preparation (see LipoMin Manual). First, SEC*-, UC- or DG-treated sample is mixed with LipoMin™ for 10 minutes. Then, captured LP (binded onto mag beads) are removed using a magnetic separator. Transfer purified EV into a new tube and is ready for downstream processing. The whole process is completed within 12 minutes.

***Our SEC protocol (for ref.)**: 2 mL plasma or serum was loaded into SEC columns followed by PBS wash until 16 sequential 1 mL eluate fractions were collected.



Mix SEC-, UC- or DG-treated sample with **LipoMin™**

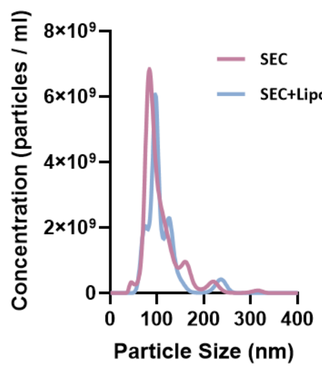
Incubate on rotator for **10 minutes**

Put magnet to remove LP (binded onto mag beads). Transfer supernatant (purified EV) to a new container for downstream processing

Results

NTA & TEM

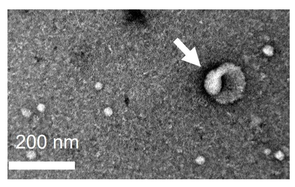
NTA and **TEM** of EVs isolated from LipoMin-treated SEC EV faction(s) show excellent concentration and EV cup-shaped morphology. (NTA: Malvern NS300 was used.)



SEC :
Conc.: 2.92x10¹¹ particles/ml
Mean: 107.0 nm

SEC + LipoMin :
Conc.: 2.26x10¹¹ particles/ml
Mean: 117.7 nm

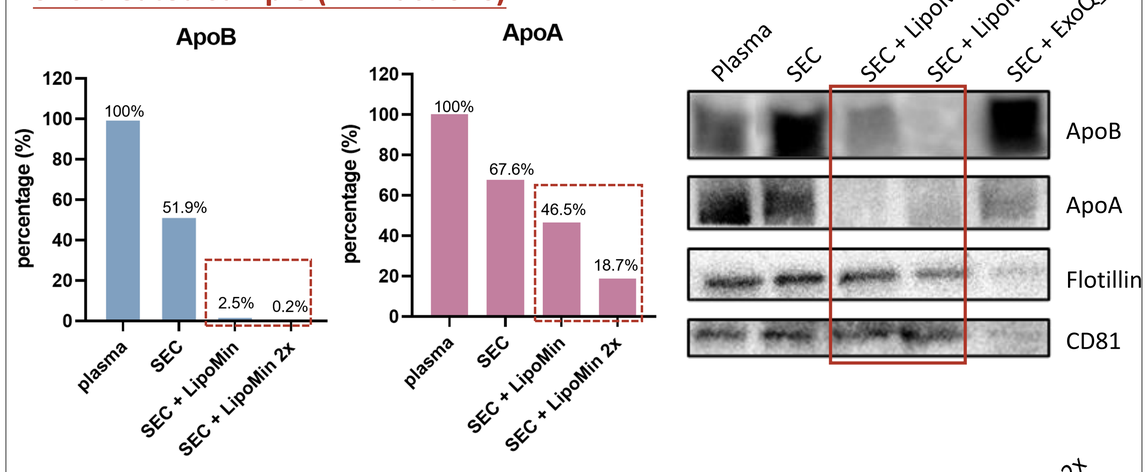
SEC + LipoMin™



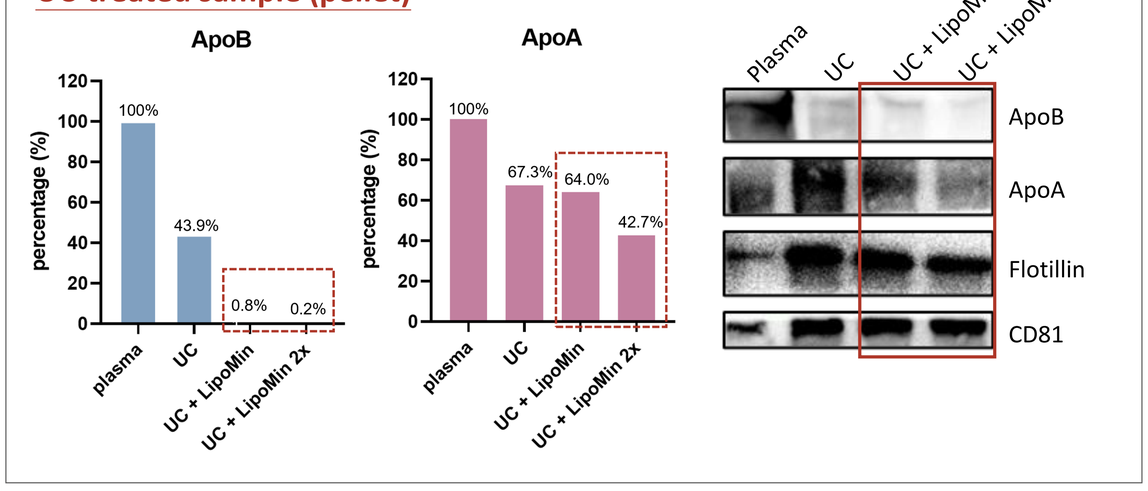
Lipoprotein Removal

Sandwich ELISA results below show LipoMin™ removes **>99.8% of ApoB** from SEC exosomal fraction(s). Substantial removal of ApoA is also demonstrated.
Note: "LipoMin" represents the suggested LipoMin™ volume in the Manual. "LipoMin 2x" denotes twice the suggested LipoMin™ volume.

SEC-treated sample (EV fractions)



UC-treated sample (pellet)



Conclusions

- LipoMin™ **depletes >80% ApoA** and **>99% ApoB** from SEC-treated plasma samples within **12 mins.**
- SEC + LipoMin™ saves >80% of operational time compares to SEC + density gradient (DG) approach.

Publication No. DAT002, Rev. 1.0