Mini-PURE-EVs Spin Column: a new platform for a fast and convenient purification of EVs and Nanoparticles from small volume amount.

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Introduction:

High-throughput sample preparation for downstream Extracellular Vesicle (EV) biomarker analysis requires scalable, affordable, fast, and easy-to-use solutions. Ultracentrifugation, density gradient centrifugation, and dead-end filtration are commonly used in the upstream processing of complex biofluids [1]. However, these methods are tedious and time-consuming, with often low sample recovery rates [2,3]. Additional pre-analytical purification steps to remove excess dye or antibodies further contribute to sample loss [4].

In this application note, we demonstrate the utility of the new MiniPURE-EVs Spin Size Exclusion Chromatography column for the quick and reproducible purification of EVs from complex samples such as plasma. Furthermore, we showcase the high efficiency of the MiniPURE-EVs Spin column in removing excess dye and antibodies prior to fluorescence analyses using Nanoparticle Tracking Analysis (NTA).

References:

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3 Clos-Sansalvador, M.; Monguió-Tortajada, M.; Roura, S.; Franquesa, M.; Borràs, F. E. Commonly Used Methods for Extracellular Vesicles' Enrichment: Implications in Downstream Analyses and Use. Eur. J. Cell Biol. 2022, 101 (3), 151227. https://doi.org/10.1016/j.ejcb.2022.151227.

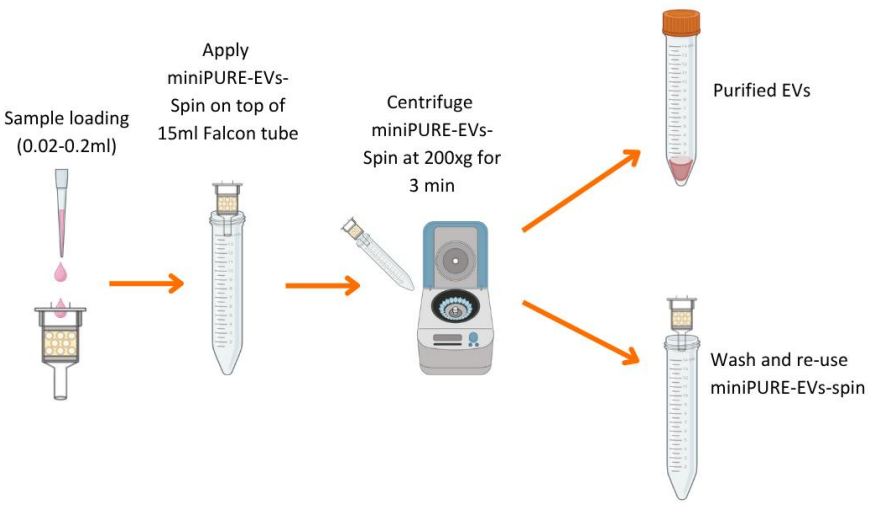
4 Rautaniemi, K.; Zini, J.; Löfman, E.; Saari, H.; Haapalehto, I.; Laukka, J.; Vesamäki, S.; Efimov, A.; Yliperttula, M.; Laaksonen, T.; Vuorimaa-Laukkanen, E.; Lisitsyna, E. S. Addressing Challenges in the Removal of Unbound Dye from Passively Labelled Extracellular Vesicles. Nanoscale Adv. 4 (1), 226–240. https://doi.org/10.1039/d1na00755f.

MiniPURE-EVs Spin: workflow and applications

Mini-PURE-EVs Spin: main applications

- EV and Nanoparticle purification from small amount of fluids (0.02 - 0.2 ml).
- No fraction collection.
- Removal of unbound membrane dyes post EV labelling.
- Removal of antibody excess post EV labelling.

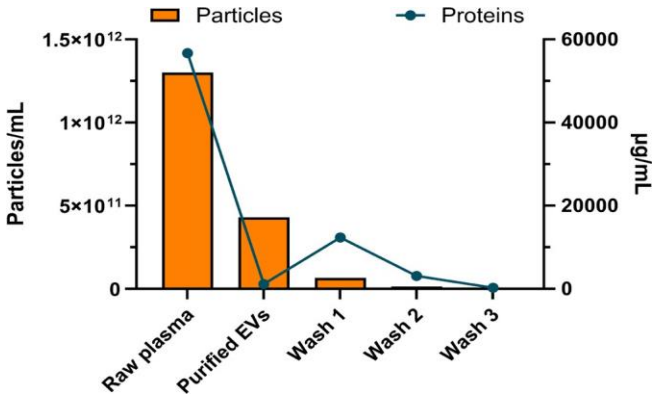
Mini-PURE-EVs Spin workflow



MiniPURE-EVs Spin: Application

- Purification of EVs from small volume amount of biofluids.

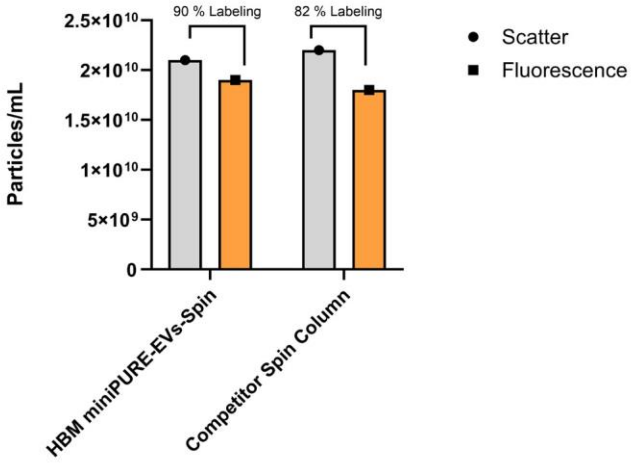
The MiniPURE-EVs Spin column was filled with 100 µL of plasma. EVs were eluted following a two-step centrifugation at 200 xg for 3 minutes each. In the first step, 100 µL of sample was processed, followed by the addition of 50 µL of PBS in the second step. The total elution volume was 150 µL, with a turnaround time of approximately 6 minutes.. After the washing steps, each filtrate was evaluated for particle number and protein amount to confirm reusability.



- EVs were successfully purified from plasma. 99 % of protein was eliminated in two sequential centrifugation steps.

- EV labelling with membrane dye: removal of dye excess with MiniPURE-EVs Spin.

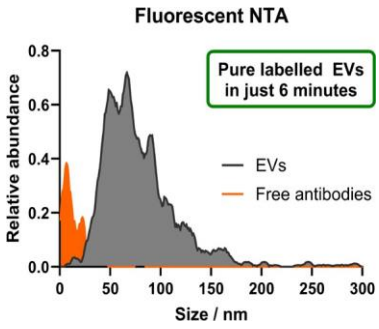
U87-derived EVs were incubated with membrane dye at 37°C for 1 hour. After incubation, a 100 µL sample was loaded onto the MiniPURE-EVs Spin column and centrifuged for 3 minutes at 200 xg. The total eluate of 100 µL, containing pure labelled EVs, was collected and analyzed with NTA in both scatter and fluorescence modes. A comparative study was performed in parallel using a competitor's spin column.

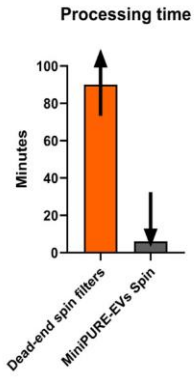


- High labelling efficiency and successful removal of unbound dye.

- EV labelling with Fluorophore-conjugated antibody: removal of dye excess with MiniPURE-EVs Spin.

COLO-derived EVs were incubated with anti-CD9 antibody (Alexa Fluor 488 conjugate) at 37°C, for 1.5h. After the incubation, the sample (100 µL) was loaded onto the MiniPURE-Evs Spin column and centrifuged for 3 minutes at 200 xg. Additional, 100 µL of PBS was loaded and centrifugation was repeated. Total eluate of 200 µL, containing pure labeled Evs, was collected and analyzed with NTA.





- Antibody-labeled EVs were successfully purified with remarkably high efficiency. More than 90% of EVs eluted in the first 200 µL, while free antibodies started eluting after 300 µL.
- Turnaround time advantage vs dead-end filtration: 6 min vs 90 min.

Conclusion:

- Fast Processing: The MiniPURE-EVs Spin column enables rapid purification of extracellular vesicles (EVs).
- High Sample Recovery: It ensures high recovery rates of EVs, maintaining sample integrity.
- Reusability: The column can be reused, offering a cost-effective solution for EV isolation.
- Versatility: Suitable for both purifying EVs from raw biofluids, cell conditioned medium and for removing excess dye.
- Ease of Use: It is significantly easier to use compared to competitor columns and dead-end filters.

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