UNIQUEST

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA
CREATE CHANGE

AVAILABLE TECHNOLOGY

A reagent for improved purity and yield of biofluid-derived extracellular vesicles (EVs)

Key Features

- Simple and effective method for improved removal of lipoproteins, major contaminants in biofluid-derived EVs.
- Improved EV yield while preserving EV morphology.
- Compatible with industry standard purification methods including TFF and SEC

Background

Extracellular vesicles (EVs) are nanosized biomolecular packages involved in intercellular communication. EVs are released by all cell types, making them broadly applicable in therapeutic and diagnostic applications. EVs are a promising next generation cell-free therapy and drug delivery platform and have potential as biomarkers for diseases diagnosis.

Sample purity is critical to maximise performance and correctly attribute observed therapeutic or diagnostic effects to EVs rather than other biological particles. Lipoprotein contaminants represent a major challenge for obtaining pure samples of biofluid-derived EVs.

Lipoproteins are six orders of magnitude more abundant than EVs in the blood circulation and overlap in size, shape, and density with EVs. These contaminants have the potential to cause:

- Lack of sensitivity in diagnostic EV assays
- Lack of efficacy in therapeutic EV development
- Inaccurate & unreliable results in mechanistic EV studies

Various methods are used to isolate EVs, including differential ultracentrifugation, density gradient ultracentrifugation, size-exclusion chromatography (SEC), tangential flow filtration (TFF), and precipitation-based isolation. Alone, all these methods are insufficient at removing lipoproteins while retaining EV yield and morphology. The current gold standard for lipoprotein removal is combining several EV isolation methods, which is a time consuming and expensive process that can negatively impact EV yield.

The technology

Researchers at the University of Queensland have discovered that a chemical reagent enhances removal of lipoproteins, improves EV yield, and preserves EV morphology. This reagent can be incorporated into existing workflows used by EV researchers and manufacturers who have difficulty efficiently removing lipoproteins from biofluid-derived EV samples.

Pre-treatment of crude plasma with the chemical, termed **"Reagent A",** prior to existing EV purification workflows allows for a simple and effective method of improving lipoprotein removal. Reagent A is hypothesised to induce lipoprotein breakdown, enabling subsequent size-based separation of the breakdown products from the EVs using industry standard methods. Pre-treatment with Reagent A provides a simpler and faster alternative compared to sequential processing using multiple instrument-based isolation techniques. Reagent A is synthetically accessible and can be stored at ambient temperature when dry.

Proof of concept

Several studies have shown that multiple EV isolation methods need to be combined to reduce lipoprotein contaminants. Table 1 shows that pretreatment with Reagent A prior to a single isolation method outperforms the current gold standard of two combined isolation methods (SEC+TFF).

Table 1. ***Levels of VLDL/LDL (ApoB) and HDL (ApoA1) lipoprotein contaminants across various purification protocols****. Measured by ELISA*

	Lipoproteins Contaminant markers (ug/10^{10} particles)				
	TFF alone	SEC alone	TFF + SEC	Reagent A + TFF	Reagent A + SEC
ApoB	3.08	2.57	0.14	0.01	0.07
ApoA1	3.50	0.55	0.07	0.05	0.12
	Other Methods			Our Methods	

See overleaf

Pretreatment of plasma with Reagent A results in improved Tangential Flow Filtration (TFF) runs:

- Allows increased flow rate by 40%
- 5-25% reduction in shear rate
- Reduces run time by 30%
- Improved yield of EVs
- Preservation of EV morphology
- Increased EV markers and reduced contaminant markers

Chemicals in the same structural class as Reagent A exhibit a similar effect, wherein pretreatment of plasma results in improved size exclusion chromatography (SEC) runs:

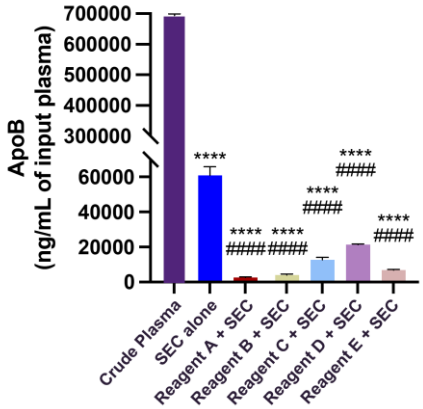

Fig 1. Level of VLDL/LDL lipoprotein contaminants, represented by ApoB marker. *Measured by ELISA. Mean ± SD of three replicates. One-way ANOVA: ****, p <0.0001 samples vs plasma; ####, p <0.0001 SEC alone vs SEC + Reagents.*

Additional data available under CDA.

Applications

- Incorporation of Reagent A into EV purification kits and workflows for research or manufacturing applications
- Conjugation of Reagent A to solid supports including resins or columns.
- Incorporation into EV biomarker-based diagnostic assays with potential for enhanced sensitivity due to improved sample purity.
- Improved purification of other plasma components – lipoproteins can interfere with plasma fractionation.

Intellectual property

An Australian provisional patent application is currently being drafted, covering methods of using Reagent A and related chemicals in lipoprotein breakdown and EV purification.

Commercialisation opportunities

UniQuest is seeking licensing or collaborative partners to further develop this exciting technology for removal of lipoproteins from plasma for EV or biomolecule purification.

Research leader

Associate Professor Joy Wolfram leads a nanomedicine and extracellular vesicle research program with the goal of developing innovative approaches that bring the next generation of treatments and diagnostics directly to the clinic. She has bridged academic and industry initiatives through her past and present board memberships and advisory roles in several companies, such as Finnish and Italian start-up companies. The Wolfram Laboratory has received funding from government and industry sources for translational research projects. Collaborators span 160 universities and industry partners across 45 countries, including a NASDAQ-listed biotech.

About UniQuest

UniQuest is the commercialisation company of The University of Queensland (UQ). In partnership with UQ researchers, we create impact through the commercialisation of UQ intellectual property (IP).

Established in 1984, UniQuest's commercialisation track record positions UQ as the leader of research commercialisation in Australasia. UniQuest has formed more than 125 start-up companies built on UQ IP. Our track record and notable successes include the blockbuster cervical cancer vaccine GARDASIL® and start-up companies Spinifex Pharmaceuticals Inc and Inflazome Ltd, which were acquired in two of the largest university start-up exits in Australian history.

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