Other

COMBINED IMMUNOPRECIPITATION AND POLYMER-BASED PRECIPITATION FOR THE ISOLATION OF SERUM-DERIVED EXOSOMES

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Exosomes are the smallest type of extracellular vesicles. They are considered messengers of the state of their parent cells. They were recently discovered as important reservoirs for biomarkers in many diseases including neurodegenerative diseases.

There is no consensus on a universal protocol for the isolation of exosomes. Ultracentrifugation is considered the gold standard protocol for their isolation. However, there is an evolving direction towards the optimization of less time-consuming protocols for the isolation of pure exosomes. Among the protocols characterized by the fast workflow are polymer-based precipitation and immunoprecipitation. This work aims at evaluating a combined immunoprecipitation and polymer-based precipitation protocol (TEI-IP) versus polymer-based precipitation protocol (TEI) for the isolation of serum-derived exosomes.

The existence of the beads (used for immunoprecipitation) hindered the physical characterization (using electron microscopy) of the TEI-IP protocol. On the other hand, instant blue staining revealed a lower degree of total protein contamination in TEI-IP relative to TEI. Both protocols displayed positive exosomal markers by western blotting (targeted). However, proteomic profiling (non-targeted) revealed exosomal enrichment and a higher degree of purity in the TEI-IP protocol relative to the TEI protocol.

Accordingly, the TEI-IP protocol is considered a promising candidate protocol for the isolation of serum-derived exosomes with a high degree of selectivity and purity relative to the TEI protocol. However, the existence of the beads hinders certain types of downstream analysis. Among the applications of this protocol is the isolation of a specific exosomal population for the measurement of novel biomarkers in neurodegenerative diseases.