**Rapid isolation of small extracellular vesicles by oligonucleotide functionalized magnetic beads and detection their surface proteins using DARPin probe**

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Small extracellular vesicles (sEVs) are a type of membrane nanocarriers that carry molecular cargo of parental cells. In addition to their exceptional role in cellular functions and communication, sEVs are a promising biomarker for diagnosing diseases in the very early stages, such as cancer and neurodegenerative disorders. In this regard, the development of a sensitive and reliable method for the isolation and detection of pathologically relevant sEVs remains a challenge. In this study, we present a method for rapid characterization of sEVs that is based on the isolation of sEVs by anti-CD63 aptamer conjugated magnetic beads followed by detecting the presence of the EpCAM and HER2 proteins on the surface of sEVs by anti-EpCAM and anti-HER2 designed ankyrin repeat proteins (DARPins). By combining magnetic capture and fluorescence identification, the method enables highly sensitive (~ 104 sEVs per mL), rapid (1 hour) and specific sEVs detection. This method provides a basis for isolating and quantifying various sEV subpopulations that contain disease specific surface-markers.

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