qEV CONCENTRATION KIT

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The qEV Concentration Kit is a particle-based concentration system for enriching purified extracellular vesicles (EVs), without the need for ultra-speed centrifugation or addition of reagents that alter the purity of EVs.

It is well known that some EV isolation methods do enrich EVs, but largely compromise EV purity. On the other hand, qEV Columns provide a rapid, reliable, standardisable system for EV isolation with high levels of EV purity – but sample concentration may be required for some downstream applications. Ultrafiltration-based EV concentration methods are commonly used, but they are imprecise as they rely on broad particle size-based separation not designed specifically for EVs. With ultrafiltration devices having a fixed minimal retrievable volume, their concentration potential is limited, and substantial EV loss can occur through EVs adhering to the filter. Alternatively, ultracentrifugation-based concentration methods might damage EV integrity, leading to a loss of EVs and their molecular cargo. Enrichment methods are therefore needed for samples separated using qEV Columns.

The qEV Concentration Kit is an all-in-one system for capturing intact EVs isolated using any of the qEV Columns producing elution volumes ranging from 600 µL to 20.0 mL. Using the qEV Concentration Kit, EVs can be concentrated to a customisable pellet, which can be used as it is for maximal EV enrichment, or resuspended in a desired volume and buffer type. The kit does not require special equipment, and does not use ultra-speed centrifugation, precipitation reagents or protease treatments.

The qEV Concentration Kit is based on Ceres Nanosciences Nanotrap® Extracellular Vesicle Particles, which are ~260 nanometre hydrogel particles made of thermostable cross-linked N-isopropylacrylamide (NIPAm) polymers previously shown to retain smaller target analytes in variable temperatures or pH conditions[1,2]. Nanotrap® EV Particles also contain chemical affinity baits on the particle surface which specifically bind with EVs (Figure 1).

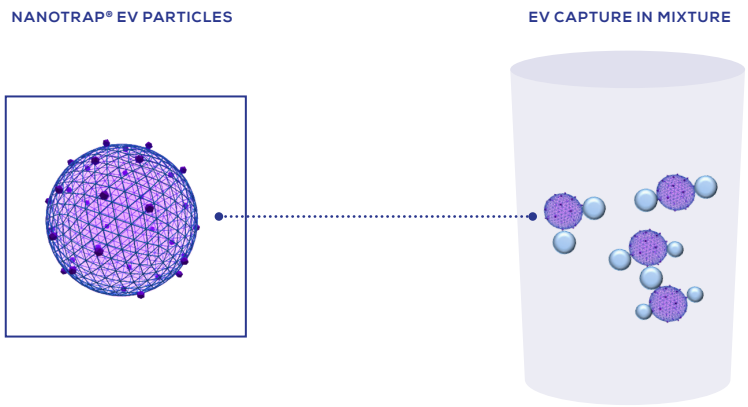

Figure 1. Structure and capture mechanism of Nanotrap® EV Particles present in qEV Concentration Kit. Nanotrap® EV Particles are ~260 nm (measured by tunable resistive pulse sensing using the Exoid) particles made of polymer functionalised with baits with high affinity to EVs.

Nanotrap® EV Particles have been functionalised with a specific chemical affinity bait that has shown a significantly higher affinity for EVs compared to other similar biological nanostructures, like enveloped viruses (Figure 2) [3,4]. Nanotrap® EV Particles are designed to irreversibly bind to the EVs. While there is no elution step available to detach intact EVs, an EV lysis step is used to release EV-associated cargo. EV binding sites are not saturated when bound to Nanotrap® EV Particles; this is relevant if they are required elsewhere, for example in other tagging or staining assays. However, users are encouraged to perform their own tagging/staining controls with Nanotrap® EV Particles to determine interference with signal intensity.

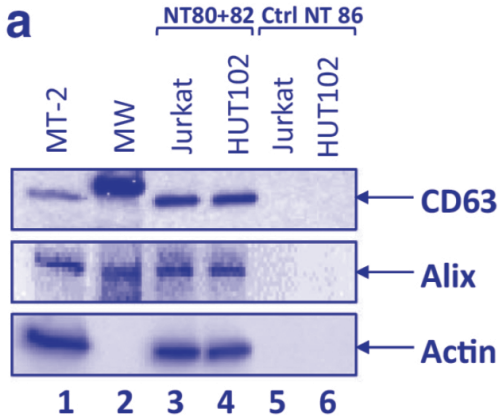

Figure 2. Nanotrap® EV Particles binding to small EVs from tissue culture supernatants. Positive exosomal markers CD63, Alix, and negative exosomal marker Actin detected by Western Blot in Jurkat (Human T cell lymphotropic virus type 1 HTLV-1 uninfected) and HUT102 (HTLV-1 infected) tissue culture supernatants. This shows that the affinity bait (NT80) in Nanotrap® EV Particles binds to exosome-type EVs, while other affinity baits specific for viruses (e.g. NT86) do not.[4]

With the qEV Concentration Kit, purified EVs are captured and highly concentrated prior to downstream analyses that do not aim to quantify EVs, but instead focus on downstream applications that look for low-abundance specific EV marker detection/quantification, molecular characterisation including RNA extraction for PCR-based assays (Figure 3) and RNA-seq studies, or protein preparations for ELISA, Western Blots and Mass Spectrometry. Importantly, alcohol-based lysis buffers that might be used for some of the aforementioned applications decrease lysis efficiency of the Nanotrap® EV Particles-bound EVs. Therefore, alcohol-free lysis buffers are recommended at this initial step. Concentrated EVs or their molecular cargo can be used for functional studies, however appropriate controls with Nanotrap® EV Particles are required to determine background effects or interference in the tested biological system.

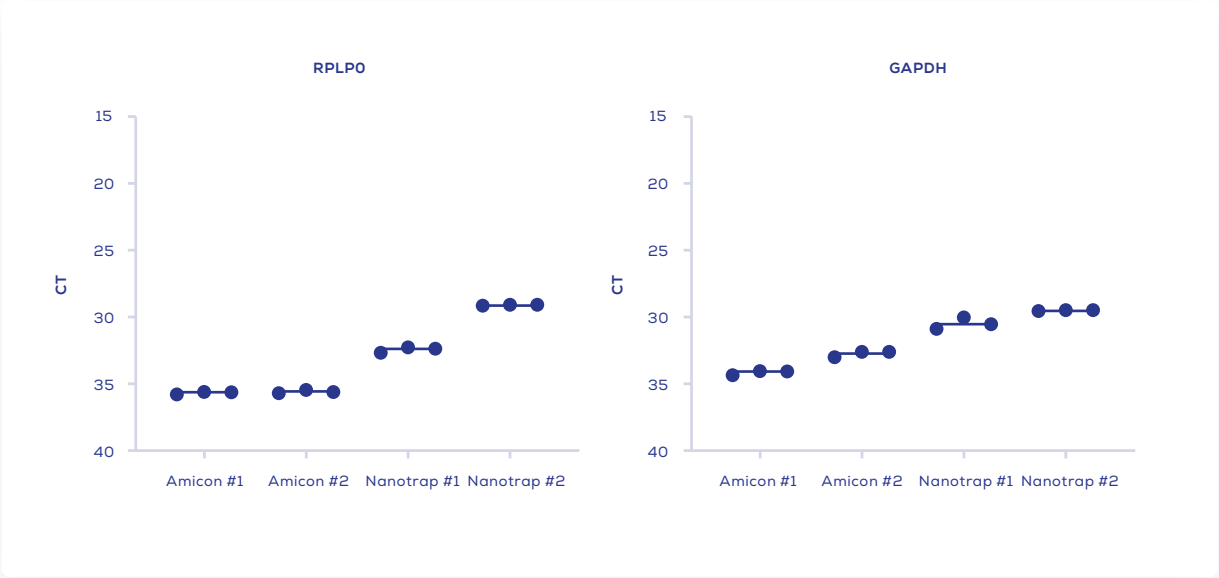

Figure 3. Comparison of the qEV Concentration Kit (Nanotrap) and ultrafiltration method (Amicon). Plasma EVs were isolated with a qEV column (qEV2, 35 nm series), concentrated with either method, resuspended in 600 µL of buffer, and used for RNA extraction with the qEV RNA Extraction Kit. mRNA targets were amplified from EV-RNA using the SYBR approach. Graphs show more mRNA targets were quantified in EVs concentrated with the qEV Concentration Kit, suggesting better performance under the same conditions. The target signal could be further improved by resuspending concentrated EVs in less buffer volume or by using the EV pellet directly with downstream application buffer.

REFERENCES

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