

# qEV CONCENTRATION KIT

## USER MANUAL



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The qEV Concentration Kit is comprised of Nanotrap® Extracellular Vesicle Particles, which are provided by Ceres Nanosciences.

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# 1 / DEFINITIONS AND WRITING CONVENTIONS

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Make sure to follow the precautionary statements presented in this guide. Safety and other special notices will appear in boxes and include the symbols detailed below.

**Table 1: Safety and Hazard Symbols**

|  |  |
|--|--|
|   | This symbol indicates general advice on how to improve procedures or recommends measures to take in specific situations. |
|  | This symbol indicates where special care should be taken.  |

**Table 2: Terminology Used in this Manual**

| TERM  | DEFINITION  |
|---|---|
| EV  | Extracellular Vesicle   |
| qEV Column  | Izon's size exclusion chromatography columns which isolate extracellular vesicles from various fluids as they pass through a column packed with porous, polysaccharide resin  |
| Buffer Volume (BV) and Purified Collection Volume (PCV) | The Buffer Volume (BV) is the discardable eluate prior to the volume containing a high proportion of purified EVs. This EV peak/EV-containing volume is known as the Purified Collection Volume (PCV). Both may differ in volume between resin types and your sample type, so optimisation around our suggested default values is encouraged. |

## 2 / SAFETY AND HAZARDS

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Refer to the Safety Data Sheet for the classification and labelling of hazards and associated hazard and precautionary statements.

The Safety Data Sheet for the qEV Concentration Kit is located at [support.izon.com/safety-data-sheets](https://support.izon.com/safety-data-sheets)

### 2.1 Hazards

If biohazardous samples are present, adhere to current Good Laboratory Practices (cGLP) and comply with any local guidelines specific to your laboratory and location regarding use and disposal.

Dispose of the following potentially contaminated materials in accordance with laboratory local, regional, and national regulations:

- ▶ Biological samples
- ▶ Reagents
- ▶ Used reaction vessels or other consumables that may be contaminated

### 2.2 Storage

Store the Nanotrap® EV Particles in the provided container between 2-8 °C. The recommended usage date is on the label located on the outside of the packaging box.



DO NOT FREEZE

### 2.3 Disposal

Waste should be disposed of in a safe manner

#### Waste Disposal Methods

- ▶ Use a licensed waste disposal company.
- ▶ Rinsed containers may be disposed of in standard solid waste stream.

## 3 / QUALITY CONTROL

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Izon products are designed and manufactured under a quality system certified to ISO 13485:2016

### 3.1 Product Use Limitations

The qEV Concentration Kit is intended for use by professional personnel only.



The qEV Concentration Kit is **not** suitable for use in conjunction with RNA extraction kits that use alcohols in the lysis buffer, as it decreases the lysis efficiency of Nanotrap® EV Particle-bound EVs. Please ensure any RNA extraction kit used downstream is compatible with this kit before use.

The Izon RNA Extraction Kit is suitable for use with this kit. Alternative kits can be used if the lysis buffer is substituted for one that does not contain alcohols, such as Microbiome Lysis Solution. Once the Nanotrap® EV Particles have been pelleted, the lysate can then be removed and used in any RNA extraction protocol.

Note that EVs bind irreversibly to Nanotrap® EV Particles. This is an important consideration for EV quantification analysis and functional studies downstream.

## 4 / INTRODUCTION TO THE qEV CONCENTRATION KIT

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EVs can be obtained from a variety of biological fluids and cell culture media. Robust, standardised and validated EV isolation techniques, such as qEV columns, produce highly pure intact EVs. However, the output volume from qEV columns might require a concentration step, depending on the downstream application.

Concentration is a means to increase the number of EVs per unit volume, with or without separation. The term “enrichment” can refer to increasing concentration, i.e. EV counts relative to volume, or to increasing EV counts/marker relative to another component.

In case of dilute biological matrices, such as urine samples, cell culture supernatants and cerebrospinal fluid samples, additional concentration steps before and/or after EV isolation may be required.

### 4.1 Overview

The qEV Concentration Kit is an all-in-one system for concentrating intact EVs isolated using qEV columns. This can be done with elution volumes ranging from 500  $\mu$ L to 20 mL. To accomplish this, the kit employs Nanotrap<sup>®</sup> Extracellular Vesicle Particles, which are provided by Ceres Nanosciences. Nanotrap<sup>®</sup> EV Particles are hydrogel particles composed of cross-linked N-isopropylacrylamide polymers that have been functionalised with chemical affinity baits.

The qEV Concentration Kit enables you to capture, irreversibly bind, and concentrate EVs before conducting downstream analyses that require highly enriched EV samples or small volumes.

The qEV Concentration Kit is comprised of 5 mL of Nanotrap<sup>®</sup> EV Capture Particles and requires a tube roller or inverter. No other specialist equipment is needed. The kit does not use precipitation reagents or protease treatments, and the concentrated EVs can be used for compatible downstream applications including RNA extraction (for PCR or RNA sequencing), Western blots or mass spectrometry. Note that particle binding is irreversible – this is an important factor when considering which downstream applications may be suitable.

The qEV Concentration Kit can be used with the qEV RNA Extraction Kit; see the qEV RNA Extraction Kit User Manual for a relevant detailed protocol.

## 5 / PROTOCOL

### 5.1 Operational Recommendations

The following recommendations are provided to ensure optimal performance of the qEV Concentration Kit:

- ▶ Perform all centrifugation steps at room temperature.
- ▶ Ensure that the centrifuge tubes used can withstand the centrifugal forces required.
- ▶ Ensure that all solutions are at room temperature prior to use.



Information on qEV column-specific sample loading volume and default PCV can be found in respective qEV column user manuals.

### 5.2 EV Concentration Procedure using Nanotrap® Particles

After qEV isolation, follow the steps below to concentrate your sample of interest.

1. Pool Purified Collection Volume of interest. For guidance on selecting your Purified Collection Volume, refer to the relevant qEV user manual.
2. The volume of Nanotrap® EV Capture Particles required to concentrate EV-containing samples is column-dependent (See [Table 3](#)). Add the appropriate volume to the entire pooled Purified Collection Volume.

**Table 3: Recommended volume of Nanotrap® EV Capture Particles to be added to Purified Collection Volume**

| qEV COLUMN USED TO ISOLATE EVs | DEFAULT PURIFIED COLLECTION VOLUME | VOLUME OF NANOTRAP® EV CAPTURE PARTICLES |
|--------------------------------|------------------------------------|--|
| qEVsingle                      | 0.68 mL                            | 50 µL                                    |
| qEVoriginal                    | 1.6 mL                             | 100 µL                                   |
| qEV1                           | 2.8 mL                             | 100 µL                                   |
| qEV2                           | 8 mL                               | 150 µL                                   |
| qEV10                          | 20 mL                              | 200 µL                                   |



3. Incubate the mixture with rotation (using a tube roller or inverter) for one hour at room temperature for efficient and specific binding of EVs to Nanotrap® EV particles.
4. Centrifuge the mixture at 16,800 x g for 10 minutes to pellet the EVs bound to Nanotrap® EV Particles.
5. Remove the supernatant being careful not to disturb the pellet containing EVs bound to Nanotrap® EV Particles.
6. The EV pellet is now ready for downstream applications. The pellet can be resuspended in a desired buffer volume or used directly with method-appropriate lysis buffer, to avoid further dilution of concentrated EVs.



If the pellet is disturbed, the sample may need to be re-centrifuged.

## 6 / RESOURCES

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### Further resources:

Visit [support.izon.com](https://support.izon.com) for application notes and typical protocols for isolating EVs from common EV samples. If you are unsure of how to prepare your sample, please contact your local support representative.



[www.izon.com](http://www.izon.com)