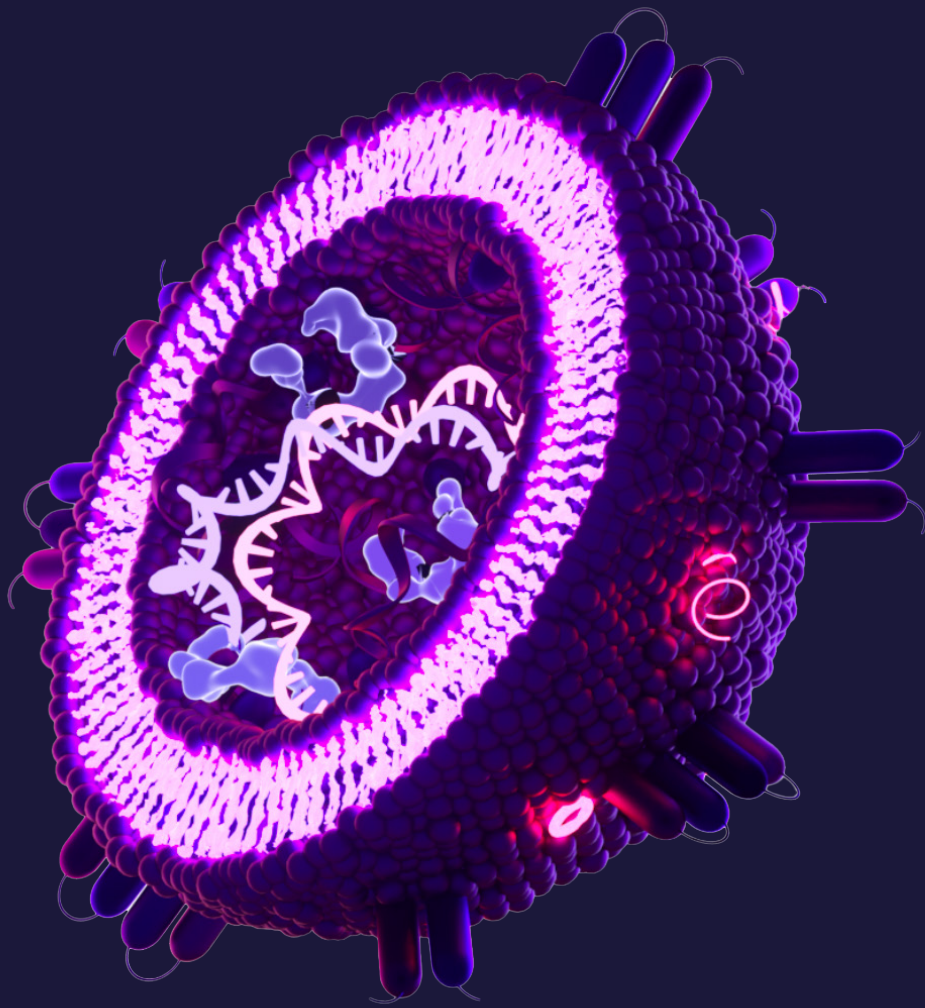


qEV MAGNETIC CONCENTRATION KIT

USER MANUAL



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The qEV Magnetic Concentration Kit is comprised of Magnetic Nanotrap® Extracellular Vesicle Particles from Ceres Nanosciences.

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TABLE OF CONTENTS

1	Terminology Used in This Manual	4
2	Safety and Hazards.....	5
2.1	Hazards.....	5
2.2	Storage.....	5
2.3	Waste Disposal	5
3	Overview	6
4	Quality Control	7
4.1	Product Use Limitations	7
4.2	Considerations for Use.....	7
5	Protocol.....	9
5.1	EV Concentration Using Magnetic Nanotrap® EV Capture Particles After qEV Isolation.....	9
6	Troubleshooting.....	10
7	Resources.....	11

1 / TERMINOLOGY USED IN THIS MANUAL

Table 1: 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 a porous, polysaccharide resin
qPCR	Quantitative polymerase chain reaction
Purified Collection Volume (PCV)	The volume immediately succeeding the Buffer Volume, containing particles of interest purified from the loaded sample. The PCV can be customised to accommodate different preferences; refer to your qEV user manual for more information.
Magnetic Nanotrap [®] EV Capture Particles	qEV Magnetic Concentration Kit reagent, functionalised to capture EVs

2/ SAFETY AND HAZARDS

2.1 Hazards

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 Magnetic Concentration Kit is located at support.izon.com/safety-data-sheets

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

Be sure to adhere to the following guidelines 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 Magnetic Nanotrap® EV Capture Particles in the container provided at 4 °C. The recommended usage date is on the label located on the outside of the packaging box.

DO NOT FREEZE.

2.3 Waste 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/ OVERVIEW

The qEV Magnetic Concentration Kit is an all-in-one system for concentrating intact EVs isolated using qEV Columns. The concentration method outlined is for the following qEV columns: qEVsingle, qEVoriginal, and qEV1, all of which are compatible with the Automatic Fraction Collector (AFC). Of these, elution volumes range from 510 μ L to 2.8 mL.

To enable EV concentration, the kit employs Magnetic Nanotrap[®] Extracellular Vesicle Particles from Ceres Nanosciences. Nanotrap[®] Particles are composed of hydrogel polymers that have been functionalised with chemical affinity baits.

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

The qEV Magnetic Concentration Kit can be used without the need for any specialised equipment, except for a magnetic rack and either a tube roller or inverter. The kit does not use precipitation reagents or protease treatments and the concentrated EVs can be used for multiple 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 Magnetic Concentration Kit can be used with the qEV RNA Extraction Kit.

Table 2: Nanotrap[®] Particles Available for Use

PARTICLE TYPE	FORMAT
Nanotrap [®] EV Capture Particles	Magnetic Nanotrap [®] Particles

Box Contents = 5 mL of Magnetic Nanotrap[®] EV Capture Particles

4/ QUALITY CONTROL

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

4.1 Product Use Limitations



The qEV Magnetic 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 EVs bound to Nanotrap® EV Capture Particles. Please ensure any RNA extraction kit used downstream is compatible with this kit before use.



This kit is suitable for use with the qEV RNA Extraction Kit. Once the Magnetic Nanotrap® EV Capture Particles have been separated, the lysate can then be removed and used for RNA extraction and analysis. Alternative RNA extraction kits can be used if the lysis buffer is substituted for one that does not contain alcohols, such as Microbiome Lysis Solution.

4.2 Considerations for Use

- ▶ EVs can be obtained from a variety of biological fluids and cell culture media. Robust, standardised and validated EV isolation techniques like qEV columns can 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 frozen human CSF samples, additional concentration steps before and/or after EV isolation may be required.

Table 3: Input and Output Volumes for qEV Columns

qEV COLUMN	INPUT qEV VOLUME	OUTPUT VOLUME (DEFAULT PCV)*
qEVsingle Gen 2	150 µL	680 µL
qEVoriginal Gen 2	0.5 mL	1.6 mL
qEV1 (Gen 2 only)	1 mL	2.8 mL
qEVsingle Legacy	150 µL	800 µL
qEVoriginal Legacy	0.5 mL	2.0 mL

*The Default Purified Collection Volume is the default Purified Collection Volume (PCV) value specific to each qEV column, selected to provide a balance of high EV recovery and purity. It is programmed on the Automatic Fraction Collector and can be adjusted; refer to your qEV user manual for more information.

5/ PROTOCOL



It is possible that a lower volume of Nanotrap® Particles (Step 3) may improve the extent of concentration, and that an even shorter incubation time (Step 4) could be sufficient. Therefore, further protocol optimisation is encouraged.

5.1 EV Concentration Using Magnetic Nanotrap® EV Capture Particles After qEV Isolation

Perform all steps at room temperature. In the interest of brevity, Magnetic Nanotrap® EV Capture Particles will be referred to as Nanotrap® Particles in this section.

1. Pool purified collection volume of interest.
2. Mix the stock Nanotrap® Particles well until there are no residual particles on the base of the stock vial. This can be achieved with gentle agitation, swirling, or using a vortex.
3. The volume of Nanotrap® Particles required to concentrate EV-containing samples will depend on the volume of sample (Table 4). Add the appropriate volume to the purified collection volume.
4. Incubate the mixture with rotation (using a tube roller or inverter) for 10 minutes at room temperature for efficient and specific binding of EVs to Nanotrap® Particles.
5. Apply a strong magnet to the side of the vial or place your sample vial into a magnetic rack to pellet the EVs bound to Nanotrap® Particles for 2 minutes.
6. With the magnet still applied, remove the supernatant, being careful not to disturb the pellet containing EVs bound to Nanotrap® Particles.
7. 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.

Table 4: Volume of Magnetic Nanotrap® EV Capture Particles Added to Specific Volumes of EV-Containing Samples

qEV COLUMN USED FOR PURIFICATION	PURIFIED COLLECTION VOLUME (mL)	VOLUME OF NANOTRAP® EV CAPTURE PARTICLES (µL)
qEVsingle Gen 2	0.51-0.85 mL	20 µL
qEVoriginal Gen 2	1.2-2.0 mL	40 µL
qEV1 (Gen 2 only)	2.1-3.5 mL	70 µL
qEVsingle Legacy	0.60-0.80 mL	20 µL
qEVoriginal Legacy	1.5-2.0 mL	40 µL

6/ TROUBLESHOOTING

If you disturb the pellet, the magnetic extraction steps ([Step 5](#) and [Step 6](#)) may need to be repeated.

If there are still EVs present in the supernatant that have not bound to the Magnetic Nanotrap® EV Capture Particles, add a fresh aliquot of Magnetic Nanotrap® EV Capture Particles (the same volume as specified in [Table 4](#)) to the supernatant and repeat the incubation and magnetic extraction steps.

7/ RESOURCES

Protocols for Isolating EVs From Common Sources

See the Izon Support Centre at support.izon.com for application notes and typical protocols for common EV samples. If you are unsure of how to prepare your sample or what volume of Magnetic Nanotrap® EV Capture Particles to add, please contact support@izon.com for assistance.

