qEV CONCENTRATION KIT

USER MANUAL

IZON

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The qEV Concentration Kit is **NOT** suitable for use in conjunction with RNA extraction kits that use alcohols in the lysis buffer, please see p7 for more information.

1 / TERMINOLOGY USED IN THIS MANUAL

Table 1: Terminology Used in this Manual

TERM	DEFINITION
EV	Extracellular vesicle
qEV Isolation Column	Izon's size exclusion columns which isolate extracellular vesicles from various fluids as they pass through a column packed with porous, polysaccharide resin
qPCR	Quantitative polymerase chain reaction
Buffer Volume	Volume of buffer eluted from the qEV Isolation Column prior to the Purified Collection Volume
Purified Collection Volume (PCV)	Volume collected post Buffer Volume containing particles of interest
Nanotrap EV Particles	qEV concentration kit reagent, functionalised to capture EVs

2 / SAFETY AND 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 qEV Concentration Kit is located at https://support.izon.com/safety-data-sheets

2.1 Hazards

qEV NanoTrap® EV capture particles are a laboratory product. However, if biohazardous samples are present, adhere to current Good Laboratory Practices (cGLP) and comply with any local guidelines specific to your laboratory and location.

For more information, see the MSDS Documentation for Izon qEV Concentration Kit columns: https://support.izon.com/safety-data-sheets

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 Nanotrap® EV Particles in the provided container at 4 °C. For stability during storage check recommended usage date in the packaging box.

DO NOT FREEZE.

2.3 Waste should be disposed of in a safe manner

Waste Disposal Methods:

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

3 / OVERVIEW

The qEV Concentration Kit is an all-in-one system for concentrating intact EVs isolated using qEV Isolation Columns in elution volumes of 600 µL to 20 mL. The kit does not require special equipment, does not use precipitation reagents or protease treatments, and the concentrated EVs can be used for compatible downstream applications including RNA extraction (PCR, RNA-seq), Western Blots or Mass Spectrometry.

Nanotrap® Extracellular Vesicle Particles are hydrogel particles made of cross-linked N-isopropylacrylamide (NIPAm) polymers functionalised with chemical affinity baits. Nanotrap® EV Particles are designed to capture, irreversibly bind and concentrate EVs prior to downstream analyses requiring highly enriched EV samples or in small volumes.[2-4]

4 / QUALITY CONTROL

In accordance with Izon's ISO 13485-certified Quality Management System, each batch of the qEV Concentration Kits is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The qEV Concentration Kit is designed for research purposes only. It is not intended for human or diagnostic use.

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. Alternate 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.

Biological Fluid

- EVs can be obtained from a variety of biological fluids, including plasma, serum, urine, cerebrospinal fluid (CSF), broncho-alveolar lavage fluid (BALF) 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 2: Different qEV columns available for use

qEV COLUMN	INPUT qEV VOLUME	OUTPUT VOLUME
qEVsingle	150 µL	600 µL
qEVoriginal - Legacy	0.5 mL	1.5 mL
qEVoriginal - Gen 2	0.5 mL	1.6 mL
qEV1	1 mL	2.8 mL
qEV2	2 mL	8 mL
qEV10	10 mL	20 mL

Table 3: Nanotrap® particles available for use

PARTICLE TYPE	FORMAT
Nanotrap® EV Capture Particles	Non-magnetic Nanotrap® particles

Box Contents = 5 mL of Nanotrap® EV Capture Particles

5 / MANUAL OPERATING INSTRUCTIONS

Notes Prior to Use

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

EV concentration using Nanotrap® particles after qEV separation/ purification

1. Pool purified collection volume of interest.
2. The volume of Nanotrap® EV Capture Particles required to concentrate EV-containing samples will depend on the volume of sample (See Table 4). Add the appropriate volume to the pooled purified collection volume.

Table 4: Volume of Nanotrap® particles added to specific volumes of EV-containing samples.

qEV COLUMN USED FOR PURIFICATION	PURIFIED COLLECTION VOLUME (PCV) (mL)	VOLUME OF NANOTRAP® EV CAPTURE PARTICLES (µL)
qEVsingle	0.6-0.8	50
qEVoriginal - Legacy	1.5-3.0	100
qEVoriginal - Gen 2	1.6-2.8	100
qEV1	2.8-4.2	100
qEV2	6.0-8.0	150
qEV10	10-20	200

3. Incubate the mixture with rotation for one hour for efficient and specific binding of EVs to Nanotrap® EV particles.
4. Centrifuge the mixture at 16,800 xg 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.

6 / TROUBLESHOOTING

If you disturb the pellet, the sample may need to be re-centrifuged.

7 / SUGGESTED PROTOCOLS FOR DOWNSTREAM ANALYSIS OF EVs CONCENTRATED WITH qEV CONCENTRATION KIT.

A / Western blot of EVs concentrated with qEV Concentration kit

1. Add minimal volume (>50 µL) of lysis buffer (e.g. Laemmli buffer with 10% Beta-mercaptoethanol) to the pellet of EVs bound to Nanotrap® EV particles to fully resuspend. Keep in mind the higher the volume added to the pellet the less concentration of EV protein that will occur.
2. Heat resuspended samples at 95°C for 3 minutes and then vortex for 5 seconds.
3. Centrifuge samples at 16,800 xg for 10 minutes to pellet only Nanotrap® EV Particles, while lysed EV contents remain in the supernatant. This will avoid loading Nanotrap® EV Particles onto gel.
4. Check that your sample has been mixed with appropriate SDS-PAGE loading buffer and load each sample (supernatant) onto gel.
5. Run gel at recommended time and voltage per manufacturer's instructions and proceed with your preferred method of Western blot.

B / Mass Spectrometry on EVs concentrated with qEV Concentration kit

1. Resuspend pellet of EVs bound to Nanotrap® EV Particles in 20 µL of 8 M urea and add 1 µL of 1 M DTT.
2. Heat sample at 50°C for 6 minutes.
3. Alkylate samples with 6 µL of 500 mM iodoacetamide in 500 mM ammonium bicarbonate and incubate for 10 minutes in dark at room temperature.
4. Dilute sample with solution of equal parts water (27 µL) and 500 mM ammonium bicarbonate (27 µL).
5. Digest samples with trypsin (1 µL) for 4 hours at 37°C.
6. Centrifuge samples at 16,800 xg for 10 minutes at room temperature to pellet only Nanotrap® EV Particles, while EV peptides remain in supernatant. Collect the supernatant and freeze overnight at -20°C to deactivate trypsin.
7. To concentrate and purify peptides in supernatant with ZipTip:
   a. Prepare ZipTip with 20 µL of Buffer B (0.1% trifluoroacetic acid + 80% acetonitrile) 3 times.
   b. Wash ZipTip with 20 µL of Buffer A (0.1% trifluoroacetic acid) 3 times.
   c. Pipette sample through ZipTip, discarding the flow-through.
   d. Wash ZipTip with 20 µL of Buffer A (0.1% trifluoroacetic acid) 3 times.
   e. Elute ZipTip with 20 µL of Buffer B (0.1% trifluoroacetic acid + 80% acetonitrile) 3 times, collecting in a clean tube.
8. Dry sample using a SpeedVac for 15 minutes.
9. Re-suspend sample in 10 µL of 0.1% trifluoroacetic acid and load on mass spectrometer.

C / RNA extraction from EVs concentrated with qEV Concentration kit.

RNA isolation from EVs purified and concentrated with qEV columns and qEV Concentration kit. **For further detailed notes, please check full user manual in qEV RNA Extraction kit.**

Prepare a working concentration of the **Wash Solution A** by adding **90 mL** of 96 - 100% ethanol (provided by the user) to the supplied bottle containing **38 mL** of concentrated Wash Solution A. This will give a final volume of **128 mL**. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

1. Add 600 µL of PBS buffer to the centrifuged pellet containing EVs bound to Nanotrap® EV Particles. Add 900 µL of Lysis Buffer A and 125 µL of Lysis Additive B.
2. Mix well by vortexing for 10 seconds then incubate at room temperature for 20 minutes.
3. Centrifuge samples for 10 minutes at 16,800 xg at room temperature to pellet only Nanotrap® EV Particles, while EV lysate remains in supernatant. Without disturbing the Nanotrap® EV Particle pellet, transfer the extracted sample supernatant into a clean tube.
4. Add 1.5 mL of 96-100% Ethanol to the supernatant sample in the clean tube and mix well by vortexing for 10 seconds.
5. Transfer 700 µL of the mixture from Step 4 into a Mini Spin column. Centrifuge for 1 minute at 3,300 xg. Discard the flowthrough and reassemble the Mini Spin column with its collection tube.
6. Repeat Step 5 as many times as necessary to transfer the remaining mixture from Step 4 into the Mini Spin column and discard the flowthrough.
7. Apply 600 µL of Wash Solution A to the column and centrifuge for 30 seconds at 3,300 xg. Discard the flowthrough and reassemble the Mini Spin column with its collection tube.

8. Repeat Step 7 one more time, for a total of two washes.
9. Spin the column empty, for 1 minute at 13,000 xg. Discard the collection tube.
10. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 50 µL of Elution Solution A to the column and centrifuge for 1 minute at 400 xg, followed by 2 minutes at 5,800 xg.
11. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 xg, followed by 2 minutes at 5,800 xg.
12. EV RNA is now ready for downstream applications.

8 / REFERENCES

1. Witwer, K. W.; Buzás, E. I.; Bemis, L. T.; et al. Standardization of Sample Collection, Isolation and Analysis Methods in Extracellular Vesicle Research. J. Extracell. Vesicles **2013**, 2 (1), 20360.
2. Van der Pol, E.; Böing, A. N.; Gool, E. L.; Nieuwland, R. Recent Developments in the Nomenclature, Presence, Isolation, Detection and Clinical Impact of Extracellular Vesicles. J. Thromb. Haemost. **2016**, 14 (1), 48–56.
3. Pleet, M. L.; Erickson, J.; DeMarino, C.; Barclay, R.A.; Cowen, M.; Lepene, B.; Liang, J.; Kuhn, J. H.; Prugar, L.; Stonier, S. W.; Dye, J. M.; Zhou, W.; Liotta, L. A.; Aman, M. J.; Kashanchi, F. Ebola Virus VP40 Modulates Cell Cycle and Biogenesis of Extracellular Vesicles, The Journal of Infectious Diseases, **2018**, 218, S365–S387
4. DeMarino, C., Pleet, M.L., Cowen, M. et al. Antiretroviral Drugs Alter the Content of Extracellular Vesicles from HIV-1-Infected Cells. Sci Rep **8**, 7653 (**2018**).

9 / RESOURCES

Protocols for EV isolation from common sources:

See Izon Support Centre http://support.izon.com for application notes and typical protocols for common EV samples. If you are unsure of how to prepare your sample, please contact support@izon.com for assistance.

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