qEV2 LEGACY
USER MANUAL

SPECIFICATIONS AND OPERATIONAL
GUIDE FOR qEV COLUMNS

RAPID & RELIABLE ISOLATION OF EXTRACELLULAR VESICLES

IZON

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1 / DEFINITIONS AND WRITING CONVENTIONS

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
Buffer volume (BV)	The volume of liquid that corresponds to the volume before the Purified Collection Volume (PCV). This volume may be different for different resin types in the same column size series.
Chromatography	A method used primarily for separation of the components of a sample. The components are distributed between two phases; one is stationary while the other is mobile. The stationary phase is either a solid, a solid-supported liquid, or a gel/resin. The stationary phase may be packed in a column, spread as a layer or distributed as a film. The mobile phase may be gaseous or liquid.
Column volume	The total volume between the upper and lower frits.
Degassing	Degassing involves subjecting a solution to vacuum to "boil" off excess dissolved gas e.g. applying a vacuum to a flask.
Flow rate	The volumetric flow in mL/min of the carrier liquid.
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, e.g., to maximise the recovery of extracellular vesicles, or to maximise protein removal.

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 Isolation columns is located at support.izon.com/safety-data-sheets

2.1 Hazards

qEV columns are a laboratory product. However, if biohazardous samples are present, adhere to current Good Laboratory Practices (cGLPs) and comply with any local guidelines specific to your laboratory and location.

Chemical Hazards

The qEV column contains < 0.1% sodium azide, which is potentially fatal if swallowed or in contact with skin. Please review the following guidelines and precautions prior to each use of the qEV column.

Prevention

1. Do not get into eyes, on skin, or on clothing.
2. Wash skin thoroughly after handling.
3. Do not eat, drink, or smoke when using this product.
4. Avoid release of product into the environment.
5. Wear protective gloves and clothing; follow general laboratory precautions.

Response

1. IF SWALLOWED: immediately call a POISON CONTROL CENTRE/Doctor.
2. IF ON SKIN: Gently wash with plenty of soap and water and immediately call a POISON CONTROL CENTRE/Doctor.
3. Remove immediately any contaminated clothing and wash before reuse.
4. Collect any spillage and dispose of appropriately.

Disposal of Biohazardous Material

Be sure to adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location regarding use and disposal.

General Precautions

- Always wear laboratory gloves, coats, and safety glasses with side shields or goggles.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious or hazardous material.
- Wash your hands thoroughly with soap and water after working with any potentially infectious or hazardous material before leaving the laboratory.
- Remove watches and jewellery before working at the bench.
- The use of contact lenses is not recommended due to complications that may arise during emergency eye-wash procedures.
- Before leaving the laboratory, remove protective clothing.
- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or physically engage with people without gloves.
- Change gloves frequently.
- Remove gloves immediately when they are visibly contaminated.
- Do not expose materials that cannot be properly decontaminated to potentially infectious or hazardous material.

- Upon completion of the tasks involving potentially infectious or hazardous materials, decontaminate the work area with an appropriate disinfectant or cleaning solution (1:10 dilution of household bleach is recommended).

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

Rapid changes in temperature should be avoided, as this can introduce bubbles into the resin bed.

Store unused qEV columns at room temperature. Used qEV columns can be stored at room temperature providing they have been cleaned according to the instructions in this document and stored in 20% ethanol or 0.05% w/v sodium azide. If the appropriate solutions are not available for storage at room temperature, then columns can be stored at +4 to +8 °C after use.

2.3 Disposal

Waste buffer should be disposed of in a safe manner. Sodium azide accumulation over time in copper pipes can result in an explosion.

3 / INTRODUCTION TO SIZE EXCLUSION CHROMATOGRAPHY

3.1 Overview

qEV Size Exclusion Chromatography (SEC) columns separate particles based on their size as they pass through a column packed with a porous, polysaccharide resin. As the sample passes through the column under gravity, smaller particles enter the resin pores on their way down and their exit from the column is delayed (Figure 1C). As the sample exits the column, sequential volumes are collected. Particles will be distributed across the volumes based on their size, with the largest particles exiting the column first and the smallest particles exiting the column last.

The packed column is equilibrated with a buffer, which fills the column. The total column volume is occupied by both the solid resin (stationary phase) and the liquid buffer (the mobile phase). As the particles do not bind to the resin, the buffer composition will not significantly affect the resolution (the degree of separation between peaks).

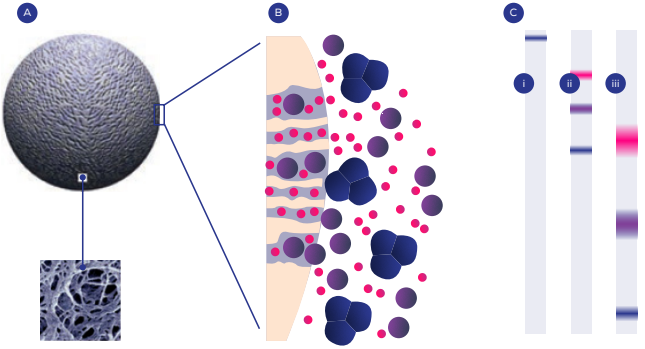

Figure 1: **Process of Size Exclusion Chromatography** (A) Schematic picture of a resin bead with an electron microscopic enlargement. (B) Schematic drawing of sample molecules diffusing into the pores of the resin beads. (C) Graphical description of separation: (i) sample is applied to the column; (ii) the smallest particles (pink) are more delayed than the largest particles (blue); (iii) the largest particles are eluted first from the column. Band broadening causes significant dilution of the particle zones during chromatography. From: GE Healthcare and Biosciences. (n.d.). Size Exclusion Chromatography Principles and Methods [Brochure]. Uppsala, Sweden. Accessed June 2019.

3.2 Intended Use

qEV columns are used to isolate extracellular vesicles from biological samples. qEV2 columns are equipped with RFID chips for use with the Automatic Fraction Collector (AFC). These chips will not impact manual use. The qEV column is intended for use in research laboratories by professional personnel for research use only. The qEV column is not intended for diagnostic purposes and should not be used to make treatment decisions.

qEV columns are designed to isolate and purify vesicles from most biological samples, including:

- Serum
- Plasma
- Saliva
- Urine
- Cerebrospinal fluid
- Cell culture media

NOTE: most 'raw' samples cannot be directly run on qEV columns and analysed with tunable resistive pulse sensing (TRPS) without some preparation such as centrifugation and concentration steps. Visit support.izon.com for recommendations and protocols.

3.3 Comparison of qEV/35 nm and qEV/70 nm Series

All qEV columns are available in one of two isolation ranges, the qEV/35 nm series and the qEV/70 nm series. The qEV/35 nm series of columns generally perform better when the target particle to be isolated is less than 110 nm in diameter, while the qEV/70 nm series of columns generally perform better when the target particle to be isolated is greater than 110 nm in diameter (see Table 3). For optimal recovery of particles between 35 and 350 nm a qEV/35 nm series column is recommended. For optimal recovery of particles between 70 and 1000 nm a qEV/70 nm series column is recommended.

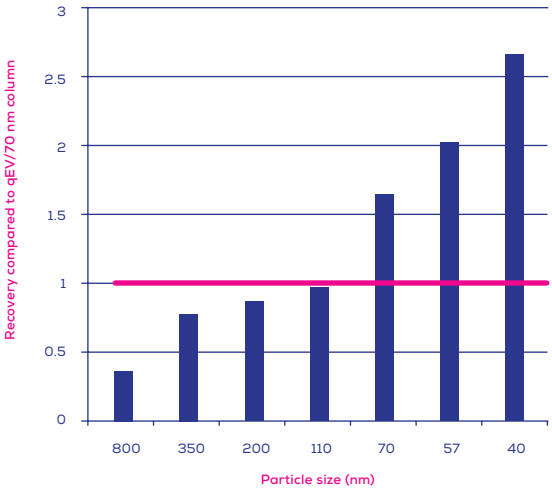

Figure 2. Particle recovery by size for qEV/35 series column compared to qEV/70 series column (pink line).

Table 3: Specifications of qEV/35 nm and qEV/70 nm Series

	qEV/35 nm SERIES	qEV/70 nm SERIES
Target Particle Size (nm)	35 nm to 350 nm	70 nm to 1000 nm
Optimum Recovery Range (nm)	Particles < 110 nm	Particles > 110 nm

3.4 qEV2 Specifications

Table 4: qEV2 Specifications

Column name	qEV2	
Column series	qEV2/35 nm	qEV2/70 nm
Optimal separation size	35-350 nm	70-1000 nm
Column volume	45.1 mL	
Sample load volume	2 mL*	
Optimal fraction size	2 mL	
Buffer volume**	14.1 mL	
Flush volume	90 mL	
Purified collection volume**	8 mL	
EV peak after buffer volume**	4 ± 2 mL	
Operational temperature	18 to 24 °C	
Buffer	PBS	
Upper, lower and loading frit size	20 µm	
pH stability working range	3–13	
pH stability cleaning-in-place (CIP)	2–14	
Shelf life (if stored correctly)	12 months	

* Loading higher sample volumes results in a lower level of purity in the later vesicle volumes, greater overlap between protein and EV elution peaks, and a higher protein peak within the PCV. Loading lower sample volumes results in a higher dilution factor of the sample. The optimal recommended sample volume for purity on the qEV2 is 2 mL.

**Values for human plasma samples only.

3.5 qEV2 Performance Characteristics

As shown in Figure 3 and Figure 4 below, particles less than 70 nm typically elute later than the PCV on the qEV/70 nm, whereas particles larger than 35 nm are captured in this zone on the qEV/35 nm column. A higher recovery in the PCV of particles larger than 70 nm occurs on the qEV/70 nm series columns compared with the qEV/35 nm series (Figure 3). Proteins typically elute slightly earlier on the qEV/35 nm series. Higher protein levels in the PCV are mainly due to an increase in EV bound proteins.

Different samples may give slightly different elution profiles and purity, hence an initial measurement of EV concentration and protein contaminants in collected fractions is recommended.

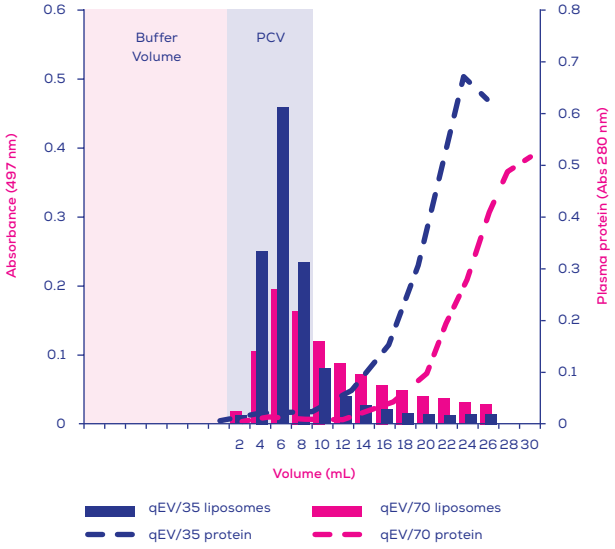

Figure 3: Comparison of plasma protein elution and recovery levels of 69 nm liposomes between a qEV2/35 nm and a qEV2/70 nm.

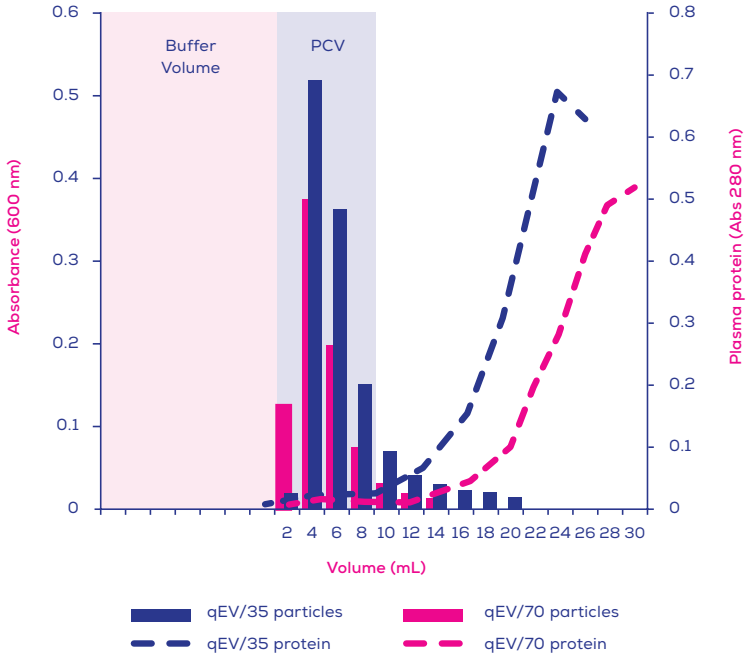

Figure 4: Comparison of plasma protein elution and recovery levels of 200 nm particles between a qEV2/35 nm and a qEV2/70 nm.

3.6 qEV2 EV Elution Profile

The elution of vesicles peak at 6 mL ± 2 mL after the buffer volume, for a 2 mL sample volume and collecting 2 mL fractions. Figure 5 shows the elution of vesicles when 2 mL of plasma sample is loaded onto a qEV2/35 nm column.

The majority of EVs typically elute in 8 mL after the buffer volume. If higher purity is desired, collect only the first 6 mL. The user therefore chooses between maximising recovery by collecting a bigger volume or maximising purity by collecting a lesser volume.

The elution of plasma protein is slower, eluting predominantly from 10 – 38 mL after the buffer volume. Any vesicles recovered beyond 8 mL contain higher protein contamination and may be less suitable for downstream analysis because of their lower purity.

Indicative protein elution profiles can be obtained by monitoring the absorbance at a wavelength of 280 nm. An accurate measurement of the level of protein can be obtained using a colourimetric protein assay.

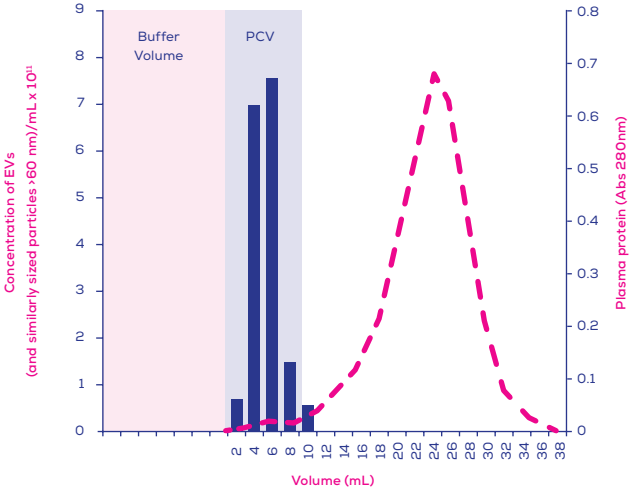

Figure 5: Typical elution profile for a qEV2/35 nm column with 2 mL of plasma loaded; proteins elute later than extracellular vesicles (EVs) and similarly sized particles >60 nm. Particle concentration was measured using TRPS and relative protein levels by absorbance at 280 nm.

4 / MANUAL OPERATING INSTRUCTIONS

The following section provides instructions for the manual use of qEV columns. For use of qEV columns with the Automatic Fraction Collector (AFC) instrument, please see the full AFC User Manual at support.izon.com

4.1 Operational Recommendations

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

Centrifuge samples prior to loading onto the column. To avoid clogging of column frits, it is recommended to filter or centrifuge the biological sample to remove large particulate matter.

- Centrifuge samples at 1,500 x g for 10 minutes to remove any cells and large particles.
- Gently move the supernatant to a new tube and centrifuge again at 10,000 x g for 10 min.
- For microvesicle isolation, use lower g-forces for the second centrifugation step.

Samples can be concentrated before application to the column or after isolation if needed. It is possible to concentrate samples before and/or after use of the qEV column, however Izon offers multiple column sizes to reduce the need for pre-analytical sample concentration. If concentration protocols are needed, please consider the following recommendations:

- Concentration of some sample types may result in the formation of precipitates and protein aggregates, especially for urine samples. Concentrated samples should be centrifuged at 10,000 x g for 10 minutes prior to loading onto a qEV column.

- Izon recommends using Amicon® Ultra Centrifugal filters (Merck C7715). Use according to manufacturer's recommendations.

Concentration of samples using filtration after purification with qEV may result in the loss of some EVs on the membrane.

Treating columns as single-use is advisable where the vesicles will be analysed for nucleic acids. This will eliminate the possibility of cross-contamination between samples.

Ensure that the sample buffer has been prepared appropriately. To maintain the functionality of EVs, the flushing buffer should be of the same temperature as the sample buffer. SEC can also be used to exchange the buffer of a sample.

- Sample buffer temperature should be within the operational temperature of 18-24 °C (65-75 °F).
- Sample buffers should be degassed and room temperature to avoid air bubbles forming in the resin bed.

Rapid changes in temperature, for example removing packed columns from a cold room and applying buffer at room temperature, can introduce air bubbles in the packed bed, resulting in poorer separation.

- Use a buffer with an ionic strength of 0.15 M or greater to avoid any unwanted ionic interactions between the solute molecule and the matrix.
- Only use freshly filtered (0.22 µm) buffer to avoid introducing particulate contamination.
- qEV columns come equilibrated in filtered PBS containing < 0.1% w/v sodium azide.

4.2 Column Setup and Equilibration

1. Equilibrate the column and the sample buffer to be within the operational temperature range of 18-24 °C.

Do not remove the column caps until the column has reached operational temperature.

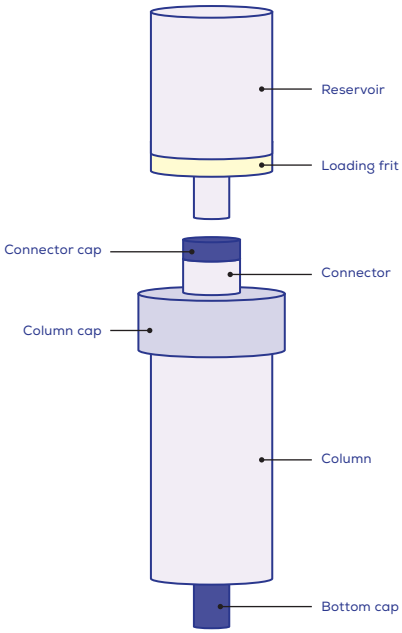

Figure 6: qEV2 setup diagram

2. Attach the column in an upright position to a stand ready for use. Alternatively, Automatic Fraction Collectors (AFCs) are available from store.izon.com
3. Rinse the reservoir with buffer.
4. Before connecting the reservoir to the column, add 5 mL of buffer to the reservoir and wait for the loading frit to wet and buffer to start running through.

If loading frit is slow to wet, apply some pressure to the reservoir top with the palm of your hand to aid the flow.

5. Allow buffer to run until it stops at the loading frit.
6. Remove the column connector cap, top up the connector with buffer, and firmly attach (a good seal is critical) the loading reservoir to the connector being careful to avoid trapping air bubbles in the connector.
7. Add buffer to the reservoir.

4.3 Column Flushing

1. Remove the bottom cap and allow the buffer to start running through the column.
2. Flush the column with at least two column volumes of PBS buffer. This will also minimise potential effects of sodium azide on your downstream applications. If an elution buffer other than PBS is to be used, equilibrate the column with at least three column volumes of the new buffer.

Only use freshly filtered (0.22 µm) buffer to avoid introducing particulate contamination.

4.4 Sample Collection

1. To avoid clogging of column frits, it is recommended to filter or centrifuge the biological sample to remove large particulate matter. See Section 4.1: Operational Recommendations for more information.
2. Continue to allow buffer to run through the column. The column will stop flowing when all of the buffer has entered the loading frit.
3. Load the prepared centrifuged sample volume onto the loading frit.

Avoid stopping the column flow during the run for long periods of time to ensure accurate EV separation.

4. Immediately start collecting the buffer volume (this includes the volume displaced by loading the sample).
5. Allow the sample to run into the column. The column will stop flowing when all of the sample has entered the loading frit.
6. Top up the column with buffer and continue to collect the buffer volume.
7. Once the buffer volume is collected, continue to collect the Purified Collection Volume (PCV).

To collect accurate volumes, only load the required volume to the top of the column, wait for the volume to run through until the flow stops and repeat.

4.5 Column Cleaning and Storage

1. After the desired fractions have been collected, the column should be cleaned and sanitised to remove residual proteins. Rinse the column with 90 mL of buffer directly after finishing fraction collection, then wash the column with 2 mL of 0.5 M NaOH, then flush with 90 mL of buffer to return the column pH to normal before loading another sample.

Simply flushing with a large volume of buffer after fraction collection is not sufficient to clean the column completely and there may be some carry-over from previous samples.

2. If storing the column for future use, it should be stored in a bacteriostatic agent such as PBS containing 0.05% w/v sodium azide, or 20% ethanol. Columns stored in 20% ethanol should be flushed with two column volumes of DI water after cleaning, then flushed with two column volumes of 20% ethanol for storage. Columns stored in buffer should be flushed with two column volumes of buffer.

Avoid adding 20% ethanol to buffer inside the column as this can precipitate salt inside the resin bed and damage the column.

3. Columns containing a bacteriostatic agent can be stored at room temperature after use, providing they have been cleaned according to the instructions above. If the appropriate storage solutions are not available then clean columns can be stored at +4 to +8 °C after use.

4.6 Restoring Column Flow After a Blockage due to Airlock in the Junction

1. Place the bottom cap on the column.
2. Remove the loading reservoir.
3. Unscrew the column cap and add buffer to the upper frit until the buffer is level with the top edge of the column.
4. Screw the column cap back on forcing buffer up through the connector junction.
5. Add 2 mL of buffer to the loading reservoir and allow buffer to run through until it stops at the loading frit.
6. Carefully attach the loading reservoir to the connector being careful to avoid trapping any air bubbles in the connector.
7. Add more buffer to the loading reservoir before removing the bottom cap.
8. The column should begin to flow again.

5 / RESOURCES

5.1 Protocols for EV Isolation from Common Sources

Visit support.izon.com for application notes and typical protocols for common EV samples. If you are unsure of what to do to prepare your sample, please contact a customer support representative via the contact tab on our website www.izon.com

5.2 EV Analysis Using TRPS

Izon recommends TRPS analysis for determination of particle size, concentration, and zeta potential. The TRPS Reagent Kit includes coating solutions for pre-coating the pore, minimising non-specific binding and provides for stable and accurate size and concentration analysis.

For TRPS analysis of the EVs, Izon recommends an initial dilution of 1/5 or 1/10 in electrolyte. Optimise the dilution to achieve a rate at the middle operating pressure of approximately 500 to 1500 particles per minute to avoid pore blockage.

Visit support.izon.com for more information on the analysis of EVs with TRPS.

5.3 qEV Concentration Kit and qEV RNA Extraction Kit

To support broader analyses downstream of qEV isolation, Izon offers the qEV Concentration Kit and qEV RNA Extraction Kit. The qEV Concentration Kit uses functionalised particles which bind irreversibly to EVs, and can be used to concentrate EVs for analysis by Western blot or ELISA, for example. The qEV RNA Extraction Kit can be used separately, or together with the qEV Concentration Kit.

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