qEV100 GEN 2

USER MANUAL

qEV100
20 nm

qEV100
35 nm

qEV100
70 nm

SPECIFICATIONS AND OPERATIONAL GUIDE FOR qEV100 GEN 2 COLUMNS

RAPID & RELIABLE ISOLATION OF EXTRACELLULAR VESICLES

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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) and Purified Collection Volume (PCV)	The Buffer Volume (BV) is the discardable eluate prior to the volume containing a high proportion of purified extracellular vesicles (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.
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.

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 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% ProClin 200 or < 0.1% sodium azide*, both of which are harmful if swallowed or in contact with skin.

*Izon is transitioning from the use of sodium azide to ProClin 200 for the storage of qEV columns. For information on how to identify which storage buffer is in your column, visit support.izon.com

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 water.
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 who are not wearing 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 upright at room temperature. Used qEV columns can be stored upright at room temperature providing they have been cleaned according to the instructions in this document and stored using a bactericide or bacteriostatic agent (e.g., PBS containing 0.05% ProClin 200 or 0.05% w/v sodium azide, or 20% ethanol). If the appropriate solutions are not available, store columns at +4 to +8 °C after use.

2.3 Disposal

Dispose of waste buffer safely and according to local guidelines. The accumulation of sodium azide in copper pipes over time 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).

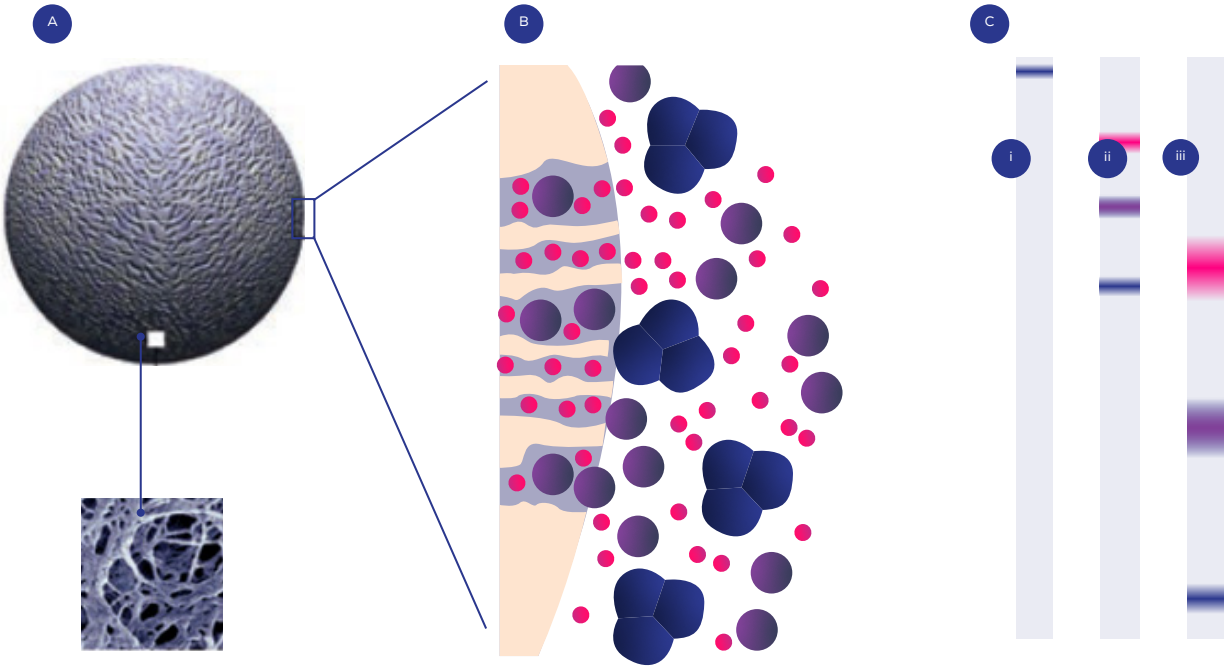


Figure 1: **Process of Size Exclusion Chromatography** (A) Schematic picture of a resin bead with an electron microscopic enlargement. (B) Schematic drawing of sample molecules diffusing into the pores of the resin beads. (C) Graphical description of separation: (i) sample is applied to the column; (ii) 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. **qEV100 columns are not designed to be used with the Automatic Fraction Collector (AFC).** qEV columns are intended for use by professional personnel only.

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 qEV100 Specifications

Table 3: qEV100 Specifications

COLUMN NAME	qEV100/20 nm	qEV100/35 nm	qEV100/70 nm
Recommended buffer volume*	210 mL	210 mL	190 mL
Recommended PCV*	100 mL		
Sample load volume**	Up to 100 mL*		
Column volume	600 mL		
Optimal fraction size	25 mL		
Flush volume	1200 mL		
Elution peak after buffer volume***	75 ± 25 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		

*These values are recommendations only and may alter with sample type/preferences. It is strongly advised that each user determines their elution profile and optimise their BV and PCV themselves.

**Larger volume columns, like the qEV100, are more affected by sample-dependent properties like EV and protein load, meaning that your optimal loading volume might be less than 100 mL. We suggest testing various loading volumes of your sample to identify when the relationship between the input volume and the output EV concentration is no longer linear. After this point, the column will be overloaded and there will be a diminishing return of EVs and decreased isolate purity.

***Based on Tunable Resistive Pulse Sensing analysis of human plasma samples.

3.4 qEV100 Performance Characteristics

When assessing the concentration of particles larger than 60 nm in the PCV of qEV100 columns, the 20 nm and 35 nm columns exhibit the highest particle recovery, followed by the 70 nm column. Among qEV100 columns, EV purity (i.e., the particle-to-protein ratio) is highest in the PCV of 35 nm and 70 nm columns, while the lowest purity is seen in 20 nm columns.

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.

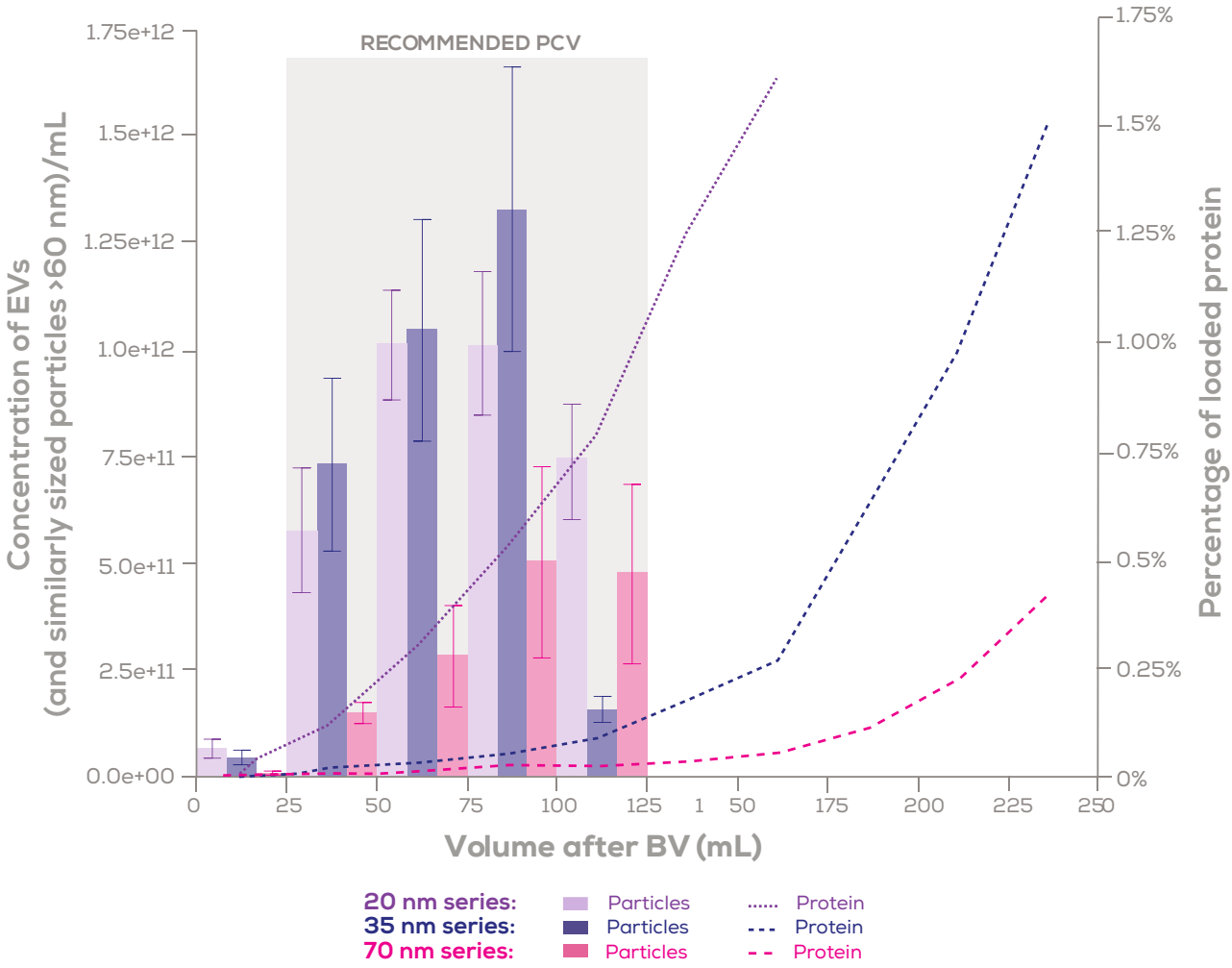


Figure 2: Elution profiles of qEV100 columns (20 nm, 35 nm, and 70 nm) with 100 mL of human plasma loaded. Particle concentration was measured using the Exoid, and protein concentration was measured using a bicinchoninic acid (BCA) assay. Particle concentration is expressed as the mean ± standard error, while the percentage of protein recovered is depicted using the mean value. n=3 for each column series. BV = Buffer Volume. PCV = Purified Collection Volume

3.5 Choosing a Purified Collection Volume

The optimal PCV will depend on the elution profile of the sample and the applications/analytical methods used downstream. Given the elution profile is impacted by sample characteristics, you should determine the elution profile of your sample to allow you to optimise the PCV.

The recommended PCV (Table 3) is based on plasma sample analysis. If you are using non-plasma samples, such as cell culture media, it is strongly recommended that you optimise your PCV based off the elution profile relevant to your samples. The fraction size should remain the same.

4 / MANUAL OPERATING INSTRUCTIONS

The following section provides instructions for the manual use of qEV100 columns.

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 transfer the supernatant to a new tube and centrifuge again at 10,000 x g for 10 minutes.
- 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), and for very large volumes, hollow fibre crossflow filtration. Use according to manufacturer's recommendations.

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

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.

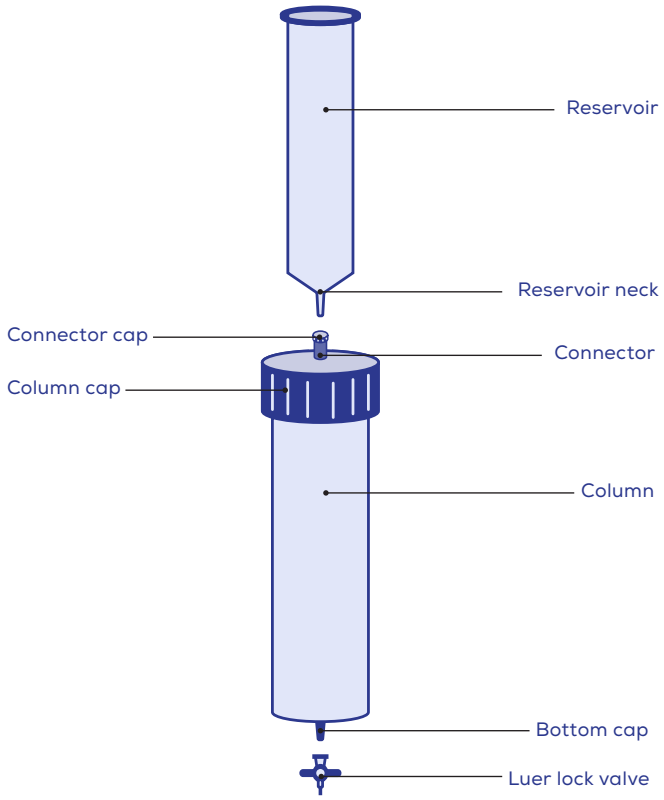
Ensure that the sample buffer has been prepared appropriately. To maintain the functionality of EVs, the flushing buffer should be of the same temperature as the sample buffer. SEC can also be used to exchange the buffer of a sample.

- 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% ProClin 200 or < 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.

2. Attach the column in an upright position to a stand ready for use.
3. Remove the bottom cap and attach the luer lock valve supplied. Ensure valve is closed (handle is horizontal).
4. Rinse the reservoir with buffer.
5. Remove the column connector cap, top up the connector with buffer, and firmly attach the reservoir to the connector (a good seal is critical) being careful to avoid trapping air bubbles in the connector.
6. Add buffer to the reservoir.

4.3 Column Flushing

1. Open the luer lock valve (handle is vertical) and allow 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 storage buffer 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. When the buffer level reaches the reservoir neck, close the valve to stop the flow.
3. Load the prepared centrifuged sample volume into the reservoir. To avoid the sample and buffer mixing in the junction, carefully pour or pipette the sample onto the inside of the reservoir wall.

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

4. Open the valve and immediately start collecting the buffer volume. The buffer volume includes the volume displaced by loading the sample.
5. Allow the sample to run into the column. Close the valve before the sample enters the connector junction.
6. Gently top up the reservoir with buffer, open the valve, and continue to collect the buffer volume.
7. Once the buffer volume is collected, continue to collect the Purified Collection Volume (PCV).
8. Use the valve to pause the flow between collected volumes.

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 1200 mL of buffer directly after finishing fraction collection, then wash the column with 200 mL of 0.5 M NaOH, then flush with 1200 mL of buffer to return the column pH to normal before loading another sample.

Flushing the column with a large volume of buffer after running your sample is not sufficient to clean the column completely and may result in carry-over from previous samples.

2. If storing for future use, store in PBS containing a bactericide or bacteriostatic agent (e.g., 0.05% w/v sodium azide or 0.05% ProClin 200), 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. Recap the column and rinse the external surface of the column, including the RFID tag, with water and thoroughly dry before storing in an upright position.

4. 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 solutions are not available then columns can be stored at +4 to +8 °C after use.

4.6 Restoring Column Flow After Airlock in the Connector Junction

1. Close the valve on the bottom of 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. Attach the reservoir to the column cap and screw the column cap and reservoir assembly back on, forcing buffer up through the connector junction and reservoir neck into the reservoir.
5. Add more buffer to the loading reservoir before opening the bottom valve.
6. 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.

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