qEV100
USER MANUAL

SPECIFICATIONS AND OPERATIONAL GUIDE FOR qEV100 COLUMNS

RAPID & RELIABLE PURIFICATION 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 in Table 1.

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
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 one is mobile. The stationary phase is either a solid, a solid supported liquid, or a gel. 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 combined volume of packed material and void volume (can be referred to as the bed volume).
Degassing	Degassing involves subjecting a solution to vacuum to "boil" off excess dissolved gas e.g. applying a vacuum to a flask.
EV zone	Indicates a particular volume collected from the column after the void volume, specified numerically for a given size. For example, 25 mL refers to the 25 mL volume collected after the void volume.
Flow rate	The volumetric flow in mL/min of the carrier liquid.
Protein zone	Indicates a particular volume collected from the column after the EV zone volume. This volume contains the majority of separated free proteins.
Void volume	The total volume of mobile phase in the column; the remainder of the column is taken up by packed gel material. It denotes the excluded volume.
Recovery rate	The percentage of vesicles that come out of the column compared with what went in.

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 http://www.izon.com/products/sds

2.1 Hazards

qEV columns 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.

Disposal of Biohazardous Material

The qEV column contains < 0.1% sodium azide, which is potentially fatal if swallowed and an irritant 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 CENTER/Doctor.
2. IF ON SKIN: Gently wash with plenty of soap and water and immediately call a POISON CONTROL CENTER/Doctor.
3. Remove immediately any contaminated clothing and wash before reuse.
4. Collect any spillage and dispose of appropriately.

For more information, see the MSDS Documentation for Izon qEV columns: https://izon.com/sds/

Sodium azide can be fatal if swallowed or in contact with skin. It can cause damage to neurological organs through prolonged or repeated exposure. It is very toxic to aquatic life with long lasting effects.

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 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 gel bed.

Store the column at 4-8 °C (39-46 °F)

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

Izon's qEV Size Exclusion Chromatography (SEC) columns separate particles based on their size as they pass through the column packed with a porous, polysaccharide resin. As particles enter the resin, smaller particles become trapped in the pores and their exit from the column is delayed (Fig 1C). As liquid exits the column, sequential fractions are collected. Particles will be distributed across the fractions 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).

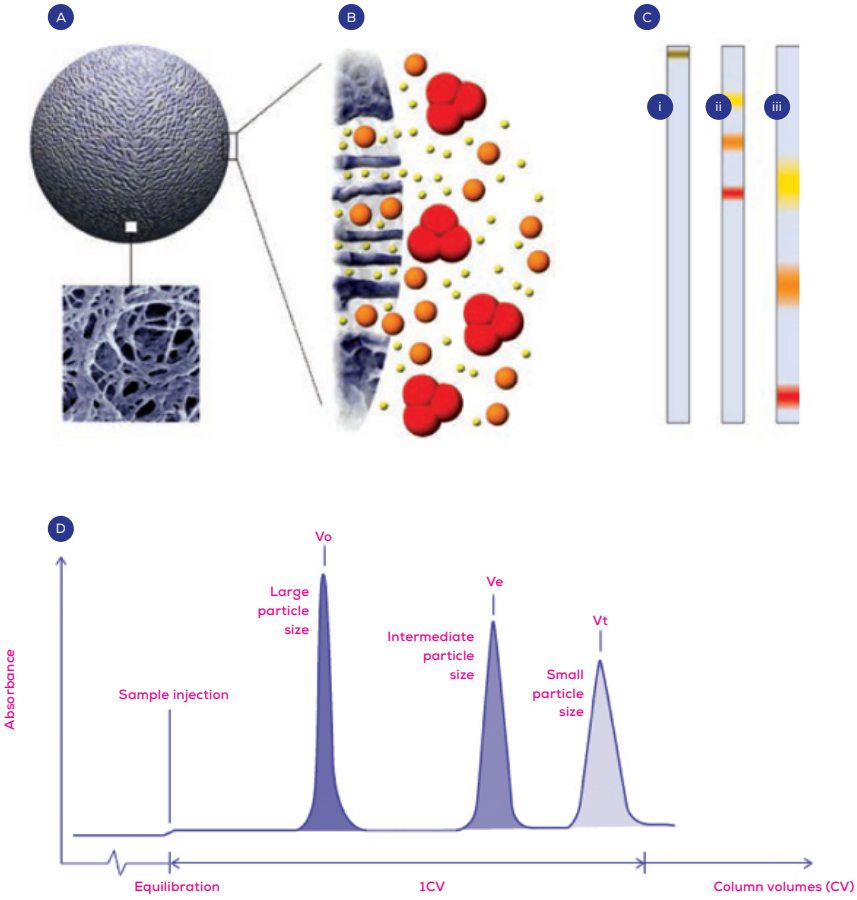

Figure 1: **Process of SEC** (A) Schematic picture of a resin bead with an electron microscopic enlargement. (B) Schematic drawing of sample particles diffusing into the pores of the resin beads. (C) Graphical description of separation: (i) sample is applied to the column; (ii) the smallest particles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

3.2 Intended Use

Izon qEV columns isolate extracellular vesicles from biological samples. **qEV100 columns are not designed to be used with the Automatic Fraction Collector (AFC).**

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

- Serum
- Plasma
- Saliva
- Urine
- Cerebrospinal Fluid (CSF)
- Cell culture media

NOTE: most 'raw' samples cannot be directly run on qEV columns and analysed with TRPS without some preparation such as centrifugation and concentration steps. Contact the Izon Support Centre for recommendations and protocols.

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

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

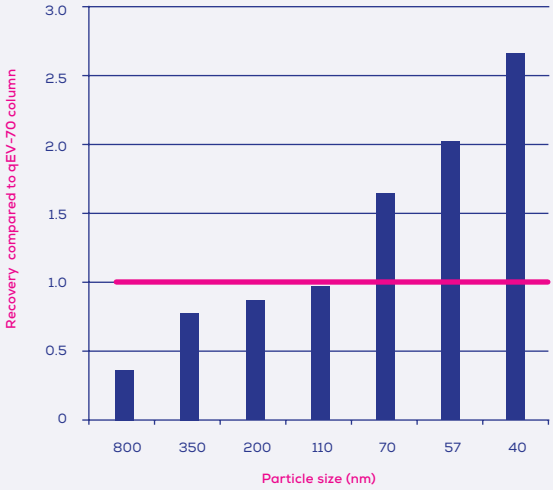

Figure 2. Particle recovery by size for qEV/35nm series column compared to qEV/70nm series column (red line).

Table 3: Specifications of qEV/30nm and qEV/70nm Series

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

3.4 qEV100 Specifications

Table 4: qEV100 Specifications

Column name	qEV100	
Column series	qEV100/70nm	qEV100/35nm
Optimal separation size	70-1000 nm	35 -350 nm
Nominal flowrate (mL/min at 20°C)	12	9
Run Time	~40 minutes	
Column volume (mL)	750	
Sample load volume (mL)	Up to 100 mL*	
Optimal fraction size (mL)	25 or 50	
Void volume (mL)	150	
Flush volume (mL)	700	
Nominal peak elution volume (mL)	200	
Elution peak after void (mL)	75 ± 25	
Operational temperature	18 to 24°C	
Buffer	PBS	
Largest size passable	1 µm	
Top and bottom filters size	20 µm	
pH stability working range	3 – 13	
pH stability cleaning-in-place (CIP)	2 – 14	
Shelf life (if stored correctly)	12 months	

* Up to 100 mL can be loaded, but for volumes higher than this the purity of some later EV fractions may be compromised.

3.5 qEV100 Performance Characteristics

As shown in Figure 3 and 4, particles less than 70 nm typically elute later than the EV zone on the qEV/70nm, whereas particles larger than 35 nm are captured in this zone on the qEV/35nm column. A higher recovery in the EV zone of particles larger than 100 nm occurs on the qEV/70nm series columns compared with the qEV/35nm series (Figure. 4). Proteins typically elute slightly earlier on the qEV/35nm series. Higher protein levels in the EV zone are mainly due to an increase in recovery of EV bound proteins.

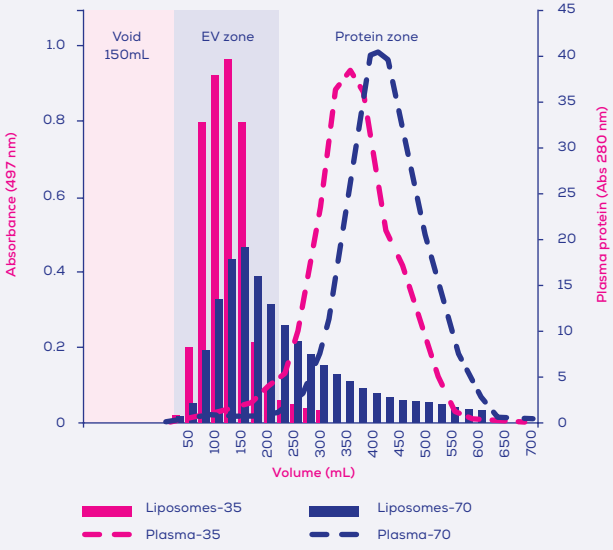

Figure 3: Comparison of plasma protein elution and recovery levels of 69 nm liposomes between a qEV100/35nm and a qEV100/70nm.

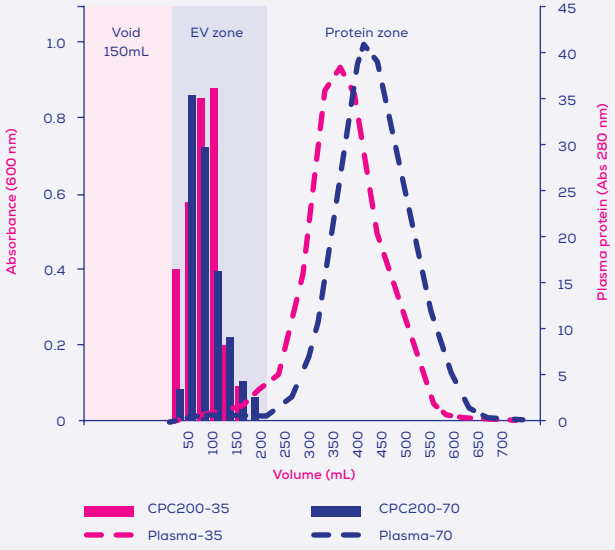

Figure 4: Comparison of plasma protein elution and recovery levels of 200 nm particles between a qEV100/35nm and a qEV100/70nm.

3.6 qEV100 EV Elution Profile

The elution of vesicles peaks at 75 mL ± 25 mL after the void volume, for a 100 mL sample volume and collecting 25 mL fractions. Figure 5 shows the elution of vesicles (80-300 nm) when 100 mL of plasma sample is loaded onto a qEV100/70nm column.

The majority of EVs typically elute in the 200 mL after the void. If higher purity is desired, collect only the first 150 mL after the void. 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 225 – 650 mL after the void volume. Any vesicles recovered beyond 225 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 colorimetric protein assay.

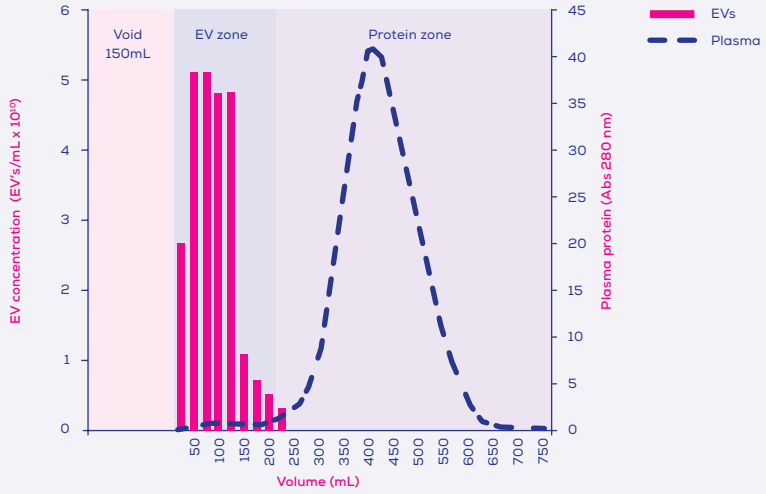

Figure 5: Typical elution profile for a qEV100/70nm column with 100 mL of plasma loaded; proteins elute in later volume than vesicles. The vesicle concentration (80-300 nm range) was measured using a qNano and relative protein levels by absorbance at 280 nm.

3.7 qEV100 Sample Input Volume Effects and Recovery Rates

3.7.1 Effect of Sample Input Volume on EV Elution Profile

Loading higher sample volumes results in a lower level of purity in the later vesicle fractions, greater overlap between protein and EV elution peaks, and a higher protein peak within the EV zone.

The optimal recommended sample volume for purity on the qEV100 is 100 mL, which consistently results in vesicles eluting in the 200 mL EV zone.

Loss of vesicles occurs with sample volumes over 100 mL, as the vesicle elution is broadened. EVs collected outside of the 200 mL EV zone are not recommended for downstream analysis where high purity is required.

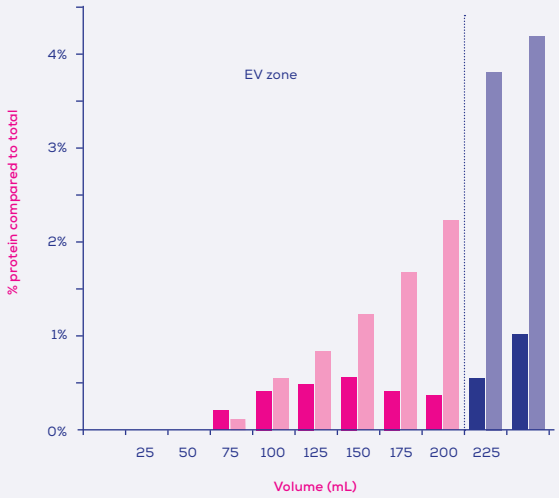

Figure 6: Percentages of protein compared with the starting quantity of protein from loading 100 mL of plasma onto a qEV100. Pink bars are the EV zone bars. qEV100/70nm bars are dark pink and dark blue. qEV100/35nm bars are light pink

3.7.2 Effect of Resin Type on Protein Elution Profile

The EV zone on a qEV100 contains very little protein, with protein levels increasing in later volumes, as shown in Figure 6. Higher protein levels for the qEV100/35nm are due in part to higher recovery of smaller EVs in the EV zone (refer to Fig. 2).

3.7.3 Effect of Sample Input Volume on EV Dilution and Recovery Rate

The recovery rate of input particles is dependent on the target particle size and the qEV column series used. Refer to the comparison between qEV/35nm and qEV/70nm series (Figures 2-4). The dilution factor depends on the sample loading volume and EV zone volume pooled.

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 qEV100 column:

- **Centrifuge samples prior to loading onto the column.** To avoid clogging of column filters, it is recommended to filter or centrifuge the biological sample to remove large particulate matter.
  - Centrifuge samples at 1,500g for 10 minutes to remove any cells and large particles.
  - Gently move the supernatant to a new tube and centrifuge again at 10,000g 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 both before and/or after use of the qEV100 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 10,000g for 10 minutes prior to loading onto a qEV column.

  - Izon recommends using Merck Millipore concentration devices (Amicon® Ultra Centrifugal filters; C7715). Centricon Plus-70 and Pellicon Tangential Flow devices. Use according to manufacturer's recommendations.
  - Concentration of samples after purification with qEV columns may result in the loss of some EVs on the membrane.

- **Single-use columns are advisable where the vesicles will be analysed for nucleic acids.**
- **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 gel 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

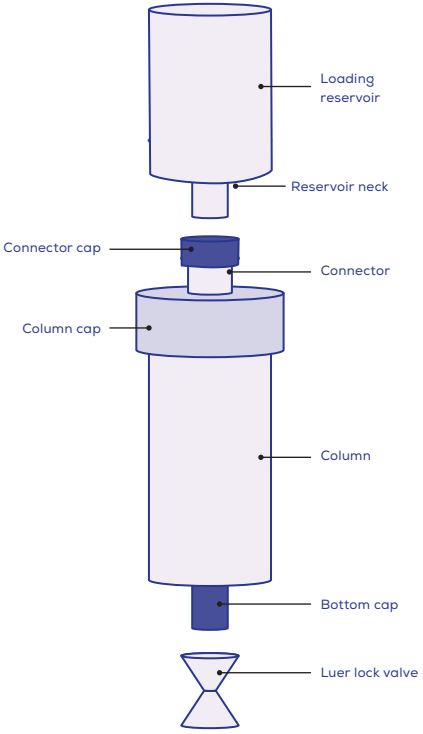

Figure 7: qEV100 setup diagram

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

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

Sample buffers should be degassed and room temperature to avoid air bubbles forming in the gel bed.

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 with the column. Ensure that the 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 (a good seal is critical) the reservoir to the connector being careful to avoid trapping air bubbles in the connector.
6. Add buffer to the reservoir.
7. Open the luer lock valve and allow buffer to start running through the column.
8. Flush the column with at least 200 mL of buffer or 2-3 column volumes if changing buffer.

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

4.3 Sample Loading

1. To avoid clogging of column filters, 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. Stop buffer flowing through the column, using the valve, when the buffer level just reaches the reservoir neck and load the prepared centrifuged sample volume into the reservoir.
3. Open the valve and immediately start collecting the void volume (this includes the sample volume).
4. Allow the sample to run into the column. Stop the flow through the column when the sample level just reaches the reservoir neck by closing the luer valve.
5. Top up the reservoir with buffer, open the valve, and continue to collect the void-volume.
6. Use the valve to switch off the flow between collected volumes if required.

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.

To ensure accurate EV separation and elution, minimise stopping the column flow during the run.

4.4 Column Flush and Storage

1. After all the desired fractions have been collected, flush the column with at least 1.5 column volumes of buffer before loading another sample.
2. If storing the column for future use, flush with buffer containing a bacteriostatic agent (e.g. 0.05 % w/v sodium azide).
3. Store the column at 4-8 °C (39-46 °F).

4.5 Restoring Column Flow After Blockage Due to an Airlock in the Junction

1. Place the bottom cap on the column or ensure the valve is switched to off position.
2. Remove the reservoir.
3. Unscrew the column cap and add buffer to the top 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. Carefully attach the reservoir to the connector, being careful to avoid trapping any air bubbles in the connector.
6. Add more buffer to the reservoir before removing the bottom cap or switching the valve to on.
7. The column should begin to flow again.

5 / RESOURCES

5.1 Column Cleaning and Sanitisation

Sanitise and remove precipitated proteins, non-specifically bound proteins and lipoproteins by washing the column with 50 mL of 0.5 M NaOH, then flush with buffer until the pH of the eluted buffer is the same as the starting wash buffer pH. The pH will return to neutral after about 2-3 column volumes of wash buffer.

5.2 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 what to do to prepare your sample, please contact support@izon.com for assistance.

5.3 EV Analysis Using TRPS

Izon recommends TRPS analysis for determination of particle size, concentration, and zeta potential. The Izon Reagent Kit includes coating solutions for pre-coating the pore, minimising non-specific binding and provides for stable and accurate sizing 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 highest operating pressure of approximately 200 to 1600 particles per minute to avoid pore blockage.

See Izon Support Centre http://support.izon.com for more information on the analysis of EVs with TRPS.

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