qEV10 GEN 2
USER MANUAL

SPECIFICATIONS AND OPERATIONAL GUIDE FOR qEV10 GEN 2 COLUMNS

RAPID & RELIABLE ISOLATION OF EXTRACELLULAR VESICLES

IZON

www.izon.com

Izon Science Ltd
PO Box 9292
Addington
Christchurch 8024
New Zealand

Telephone: +64 3 357 4270
Email: support@izon.com
Website: www.izon.com

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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 one 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.
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 www.izon.com/resources

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.

Disposal of Biohazardous Material

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.

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

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 contact 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.

Unused qEV columns can be stored 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 molecules enter the resin, smaller particles become trapped in the pores and their exit from the column is delayed (Figure 1C). As liquid 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).

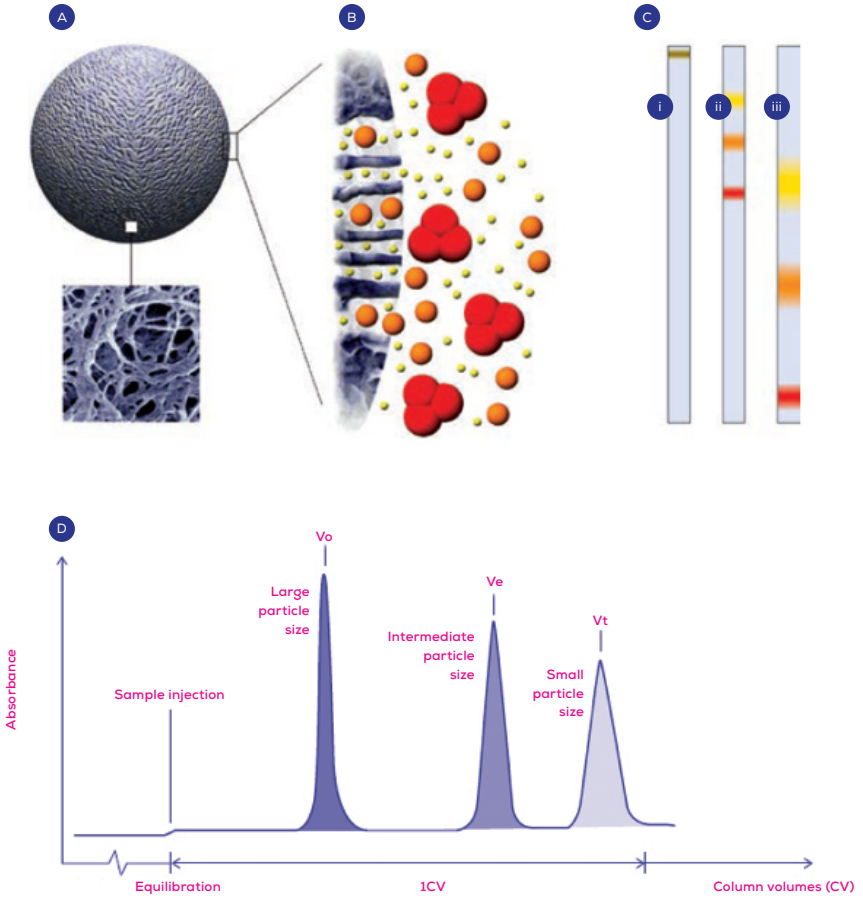

Figure 1: **Process of Size Exclusion Chromatography** (A) Schematic picture of a resin bead with an electron microscopic enlargement. (B) Schematic drawing of sample molecules diffusing into the pores of the particle. (C) Graphical description of separation: (i) sample is applied to the column; (ii) the smallest particles (yellow) are more delayed than the largest particles (red); (iii) the largest particles are 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. qEV10 Gen 2 columns are equipped with RFID chips for use with the Izon Automatic Fraction Collector (AFC). **These chips will not impact manual use.** The column is intended to be used 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 (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, support.izon.com, for recommendations and protocols.

3.3 qEV10 Gen 2 Specifications

Table 3: qEV10 GEN 2 Specifications

Column name	qEV10 GEN 2	
Column series	qEV10/70 nm GEN 2	qEV10/35 nm GEN 2
Optimal Separation Size	>110 nm	<110 nm
Buffer volume**	22.9 mL	23.2 mL
Sample load volume	Up to 10 mL*	
Column volume	69.3 mL	
Optimal fraction size	5 mL	
Flush volume	140 mL	
PCV**	20 mL	
Elution peak after buffer volume**	15 ± 5 mL	
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	

*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 qEV10 Gen 2 is 10 mL.

**Values based off analysis of human plasma samples.

3.4 qEV10 Gen 2 Performance Characteristics

A higher recovery of particles larger than 60 nm was observed in the PCV of qEV/35 nm columns than in that of the qEV/70 nm columns (Figure 2). Proteins typically eluted slightly earlier on qEV/35 nm columns.

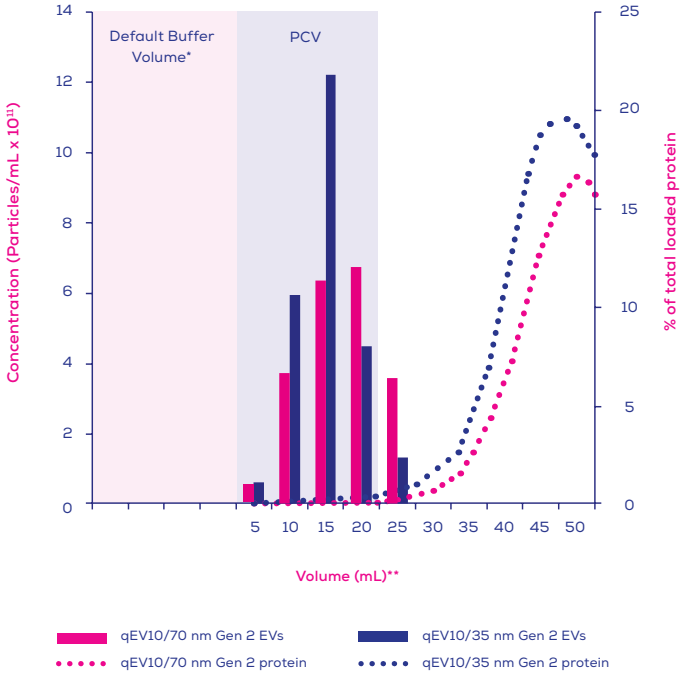

Figure 2: Comparison of total protein elution levels and concentration of extracellular vesicles and similarly sized particles >60 nm between qEV10/35 nm Gen 2 and qEV10/70 nm Gen 2 columns with 10 mL of human plasma loaded, normalised for buffer volume. EV concentration was measured using an Exoid and protein levels by bicinchoninic acid (BCA) assay.

*Nb: the default buffer volume values differ for qEV10/35 nm Gen 2 (23.2 mL) and qEV10/70 nm Gen 2 (22.9 mL) columns.

**Volumes are labelled as the highest volume in that sample i.e label "5" refers to the volume from 0.0-5.0 mL after the buffer volume, label "10" refers to the volume from 5.0-10.0 mL after the buffer volume and so on.

3.5 qEV10 Gen 2 Elution Profile

The elution of vesicles typically peaks at 15 mL after the buffer volume, for a 10 mL sample volume and collecting 5 mL fractions. Figure 3 shows the elution of vesicles when 10 mL of plasma sample is loaded onto a qEV10/35 nm Gen 2 column.

The majority of EVs typically elute in 20 mL after the buffer volume. If higher purity is desired, collect only the first 15 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 25 – 70 mL after the buffer volume. Any vesicles recovered beyond 20 mL contain higher protein contamination and may be less suitable for downstream analysis because of their lower purity.

Protein elution profiles can be obtained by performing bicinchoninic acid (BCA) assay analysis on individual fractions, or an alternative colorimetric protein assay.

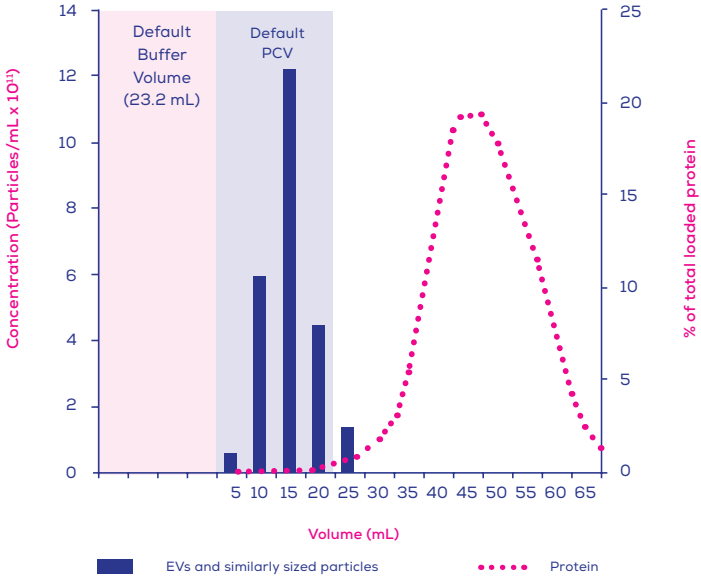

Figure 3: Typical elution profile for qEV10/35 nm Gen 2 columns with 10 mL of human plasma loaded; proteins elute in a later volume than extracellular vesicles (EVs) and similarly sized particles >60 nm. The vesicle concentration was measured using an Exoid and protein levels by bicinchoninic acid (BCA) assay.

3.6 Choosing a Purified Collection Volume

The optimal PCV will depend on the elution profile of the sample and the applications/analytical methods used downstream. This section makes recommendations based off data collected from qEV10 Gen 2 columns with 10 mL of human plasma loaded; other sample types may differ slightly in elution profile. There are several different optimisation scenarios that may be applicable for specific sample collection (Table 4). The default recommended PCV is a balance between EV recovery and high purity.

Table 4: Recommended parameters for different optimisation scenarios

OPTIMISATION SCENARIO	ADJUSTED BUFFER VOLUME		PCV
	70 nm Series	35 nm Series	
EV Concentration (3.6.1)	27.9 mL	28.2 mL	15 mL (3 x 5 mL)
EV Recovery (3.6.2)	22.9 mL	23.2 mL	35 mL (7 x 5 mL)
Maximum Purity (3.6.3)	22.9 mL	23.2 mL	15 mL (3 x 5 mL)
Default*	22.9 mL	23.2 mL	20 mL (4 x 5 mL)

*These are the default settings when using the Automatic Fraction Collector, which will need to be changed when optimising for EV recovery, concentration or maximum purity scenarios.

3.6.1 Optimised for EV Concentration

On average the highest concentration of EVs and similarly sized particles occurs in the volume 10-15 mL after the defined buffer volume (Figure 2). This can vary from sample to sample, possibly peaking at the volumes 5-10 mL or 15-20 mL after the buffer volume. To maximise EV concentration from an unknown sample, Izon recommends collecting the entire volume from 0-15 mL after the buffer volume to accommodate for this possible shift in peak. To achieve this the adjusted buffer volume should be set to 27.9 mL for the 70 nm series, or 28.2 mL for the 35 nm series.

3.6.2 Optimised for EV Recovery

To collect the majority of EVs, Izon recommends collecting up to 35 mL after the defined buffer volume (22.9 mL for the 70 nm series, or 23.2 mL for the 35 nm series). It should be noted that this volume will contain higher levels of protein than is normally recommended for downstream applications such as TRPS, which has a protein concentration limit of 200–300 µg/mL before measurements become difficult due to the high protein levels. Pooling these samples can mitigate this challenge.

3.6.3 Optimised for EV Purity

To collect a significant number of the EVs with a high level of purity, Izon recommends collecting only the first 15 mL after the defined buffer volume (22.9 mL for the 70 nm series, or 23.2 mL for the 35 nm series). This keeps the amount of protein in the sample to a minimum whilst still collecting a significant portion of the EVs in the sample.

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 filters, 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 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 both 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 Merck Millipore concentration devices (Amicon® Ultra Centrifugal filters; C7715). Use according to manufacturer's recommendations.
- Concentration of samples 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 at room temperature to avoid air bubbles forming in the gel/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

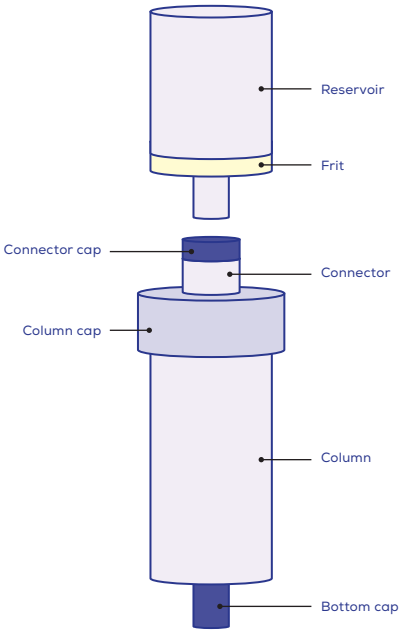

Figure 4: qEV10 Gen 2 setup diagram.

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

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

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

2. Attach the column in an upright position to a stand ready for use.

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 frit to wet and buffer to start running through.
   - If 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 frit.

6. Remove the column connector cap, top up the connector with buffer, and firmly attach the loading reservoir to the connector being careful to avoid trapping air bubbles in the connector (a good seal is critical).

7. Add buffer to the reservoir.

8. Remove the bottom cap and allow the buffer to start running through the column.

9. If an elution buffer other than 1X PBS is to be used, equilibrate the column with at least 3 column volumes of new buffer. This step is optional if using the same buffer the column comes in (1X PBS). In this case, one column volume of PBS buffer may be flushed through the column before moving to sample loading, if desired.

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. 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.

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.

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.

4.4 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 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. Add 2 mL of buffer to the loading reservoir and allow buffer to run through until it stops at the 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.

4.5 Column Cleaning and Storage

1. After all the desired fractions have been collected follow the cleaning protocol outlined subsequently, before loading another sample.
2. If storing the column for future use, perform the cleaning procedure with buffer containing a bacteriostatic agent (e.g. 0.05 % w/v sodium azide) or 20% ethanol.
3. Columns 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.

5 / RESOURCES

5.1 Column Cleaning and Sanitisation

To sanitise and remove precipitated proteins, non-specifically bound proteins, and lipoproteins, Izon recommends cleaning the column with 140 mL of buffer, followed by loading 20 mL of 0.5 M NaOH, followed by another 140 mL of buffer to return the column pH to normal. Allow all fluid to flow into the frit before loading the next amount. 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.

5.2 Protocols for EV Isolation from Common Sources

See Izon Support Centre 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 TRPS 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 1500 particles per minute to avoid pore blockage.

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

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