QUICK START GUIDE

FOR qEV2 LEGACY COLUMNS (35 nm & 70 nm)

This quick start guide provides general operating instructions. For more detailed information, you can download the full library of qEV User Manuals and other resources from the Izon support portal at support.izon.com

Safety Data Sheets are available at support.izon.com/safety-data-sheets

The qEV column contains < 0.1% sodium azide, which is potentially fatal if swallowed or in contact with skin. Please refer to the user manual for more information.

STORAGE BEFORE USE

Store unused qEV columns at room temperature.

INTENDED USE

qEV columns are used to isolate extracellular vesicles from biological samples and are equipped with RFID chips for use with the Automatic Fraction Collector (AFC). These chips will not impact manual use.

qEV columns are intended for use in research laboratories by professional personnel for research use only. qEV columns are not intended for diagnostic purposes and should not be used to make treatment decisions.

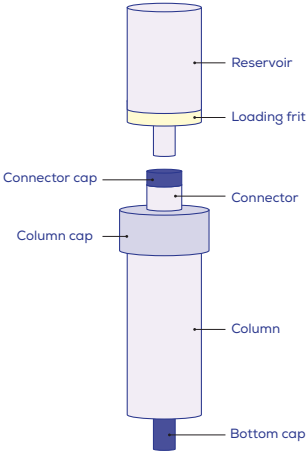
OPERATIONAL RECOMMENDATIONS

1. Centrifuge samples prior to loading the column to remove cells and large cellular debris. Initially centrifuge at 1,500 x g for 10 minutes to remove any cells and large particles. Re-centrifuge the supernatant at 10,000 x g for 10 minutes.
2. For large volume samples, it is possible to concentrate the sample before loading onto the qEV column. Izon recommends using Amicon® Ultra Centrifugal filters (Merck) and for very large volumes, hollow fibre crossflow filtration. This is not applicable for serum and plasma samples, which have very high levels of protein.
3. Izon recommends single use of columns if you intend on analysing vesicles for nucleic acids.
4. Ensure the sample buffer is the same temperature as the column (preferably 18-24 °C).
5. **Only use freshly filtered (0.22 µm) buffer to avoid contamination.**

OPERATING INSTRUCTIONS

qEV2 COLUMN SPECIFICATIONS

Sample Load Volume	2.0 mL
Column Volume	45.1 mL
Buffer Volume	14.1 mL
Optimal Fraction Size	2.0 mL
Buffer required per sample collection	25.0 mL



EQUILIBRATION

1. Equilibrate the column and the sample buffer to be within the operational temperature range of 18-24 °C. **Do not remove column caps until the operational temperature range is reached.**
2. Attach the column in an upright position to a stand ready for use. Automatic Fraction Collectors (AFCs) are available from store.izon.com
3. Rinse the reservoir with buffer.
4. Add 5 mL of buffer to the reservoir and wait for the loading frit to wet and buffer to start running through. Apply pressure to the top with the palm of your hand if required.
5. Allow buffer to run until it stops at the loading frit.
6. Remove the connector cap, top up the connector with buffer, and firmly attach the 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.

COLUMN FLUSHING

1. Remove the bottom cap 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 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. The column will stop flowing automatically when all of the buffer has entered the loading frit.

MANUAL SAMPLE COLLECTION

1. Filter or centrifuge the biological sample to remove large particulate matter. Refer to operational recommendations above.

2. Once buffer has stopped flowing into the column from flushing, 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.

3. Immediately start collecting the buffer volume[1] (this includes the volume displaced by loading the sample).
4. Allow the sample to run into the column. The column will stop flowing when all of the sample has entered the loading frit.
5. Top up the reservoir/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.

6. Once the buffer volume is collected, continue to collect the Purified Collection Volume (PCV)[2]. Refer to Figures 1 and 2.

COLUMN CLEANING AND STORAGE

1. After the desired volumes have been collected, flush the column with 90 mL of buffer, followed by 2 mL of 0.5 M sodium hydroxide (NaOH), followed by another 90 mL of buffer before loading another sample.
2. If storing for future use, the column 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 solutions are not available then columns can be stored at 4-8 °C after use.

RESTORING COLUMN FLOW AFTER AIRLOCK IN THE CONNECTOR JUNCTION

1. Place the bottom cap on the column.
2. Remove the reservoir.
3. Unscrew the column cap and add buffer to the column 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 reservoir and allow buffer to run through until it stops at the loading frit.
6. Carefully attach the reservoir to the connector being careful to avoid trapping any air bubbles in the connector.
7. Add more buffer to the reservoir before removing the bottom cap.

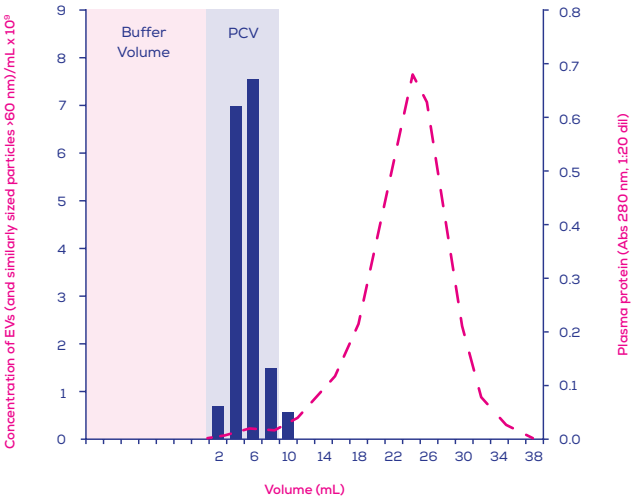


Figure 1. Typical elution profile for a qEV2/35 nm column with 2 mL of plasma loaded; proteins elute in later volumes than extracellular vesicles (EVs) and similarly sized particles >60 nm. Particle concentration was measured using TRPS and relative protein levels by absorbance at 280 nm.

EVs and similarly sized particles>60 nm

Protein absorbance

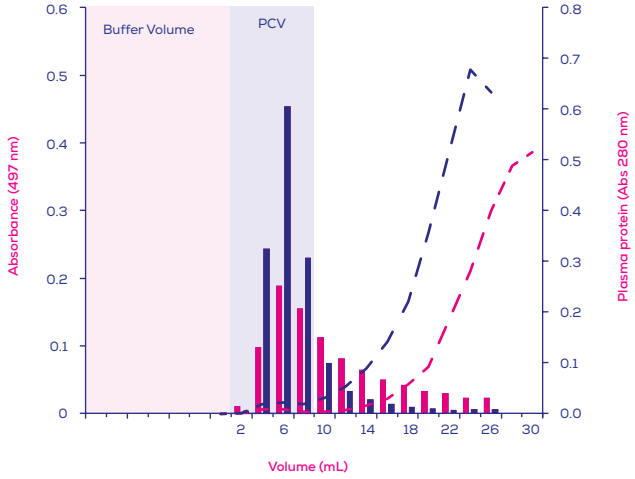


Figure 2. Comparison of plasma protein elution and recovery levels of 69 nm liposomes between a qEV2/35 nm and a qEV2/70 nm.

qEV/70 liposomes

qEV/70 protein

qEV/35 liposomes

qEV/35 protein

[1] 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.
[2] 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.