QUICK START GUIDE

FOR qEV10 GEN 2 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

INTENDED USE

qEV columns 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 to be used 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.

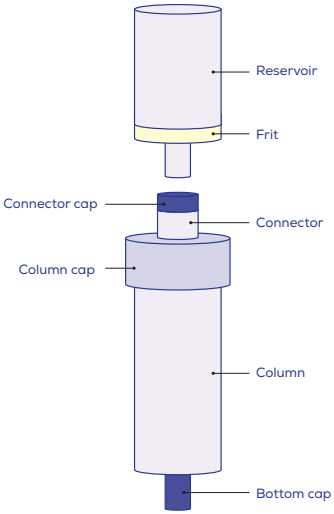
OPERATIONAL RECOMMENDATIONS

1. Centrifuge samples prior to loading the column to remove cells and large cellular debris. Initially centrifuge at 1500 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. This is not applicable for serum and plasma samples, which have very high levels of protein. Izon recommends using Merck Millipore concentration devices, Amicon® Ultra Centrifugal filters and for very large volumes, hollow fibre crossflow filtration.
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 between 18-24 °C).
5. **Only use freshly filtered (0.22 µm) buffer to avoid contamination.**

OPERATING INSTRUCTIONS

qEV10 GEN 2 COLUMN SPECIFICATIONS

Sample Load Volume	≤ 10 mL
Column Volume	69.3 mL
Buffer Volume	22.9 mL (70 nm) 23.2 mL (35 nm)
Optimal Fraction Size	5.0 mL
Buffer required per sample collection	50.0 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 are available from Izon Science.
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 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 one column volume of buffer. If your downstream applications are affected by sodium azide, flush with at least 2 column volumes of buffer. If an elution buffer other than PBS is to be used, equilibrate the column with at least 3 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.
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 (BV)[1]. The BV 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 FLUSH AND STORAGE

1. After the desired volume has been collected, flush the column with 140 mL of buffer, followed by 20 mL of 0.5 M Sodium Hydroxide (NaOH), followed by another 140 mL 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) 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 4-8 °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 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 reservoir and allow buffer to run through until it stops at the 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.

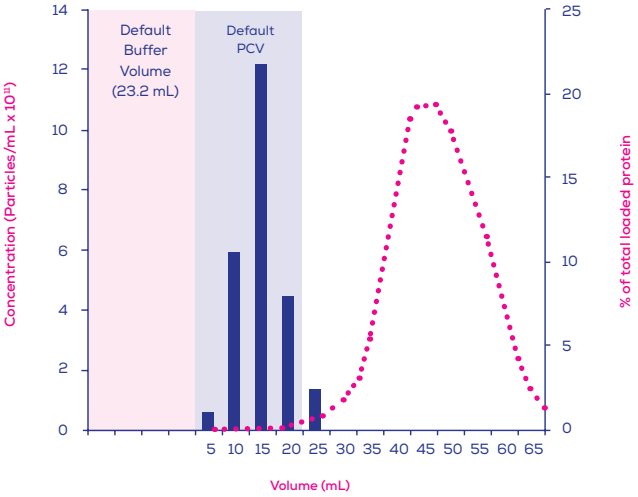


Figure 1. Typical elution profile for a qEV10 35 nm Gen 2 column with 10 mL of human plasma loaded; proteins elute in a later volume than extracellular vesicles (EVs) and similarly sized particles >60 nm. The EV concentration was measured using an Exoid and protein levels by BCA analysis.

EVs and similarly sized particles
Protein

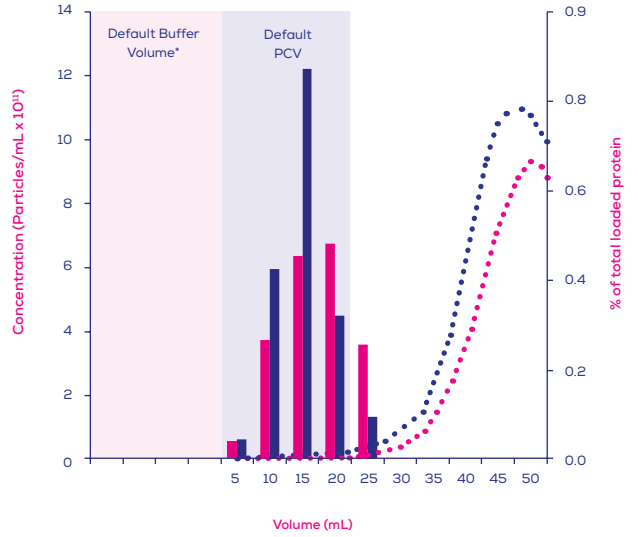


Figure 2. Comparison of total protein elution levels and concentration of extracellular vesicles (EVs) and similarly sized particles >60 nm between qEV10/35 nm Gen 2 and qEV10/70 nm Gen 2 columns with 10 mL of human plasma loaded, normalised for buffer volume. EV concentration was measured using an Exoid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

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

qEV/70 EVs and similarly sized particles
qEV/70 protein levels
qEV/35 EVs and similarly sized particles
qEV/35 protein levels

[1] Buffer volume (BV): volume of buffer that elutes from the column before the particles of interest.
[2] Purified Collection Volume (PCV): volume purified by the column containing the particles of interest.