QUICK START GUIDE

FOR qEV100 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

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. 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.

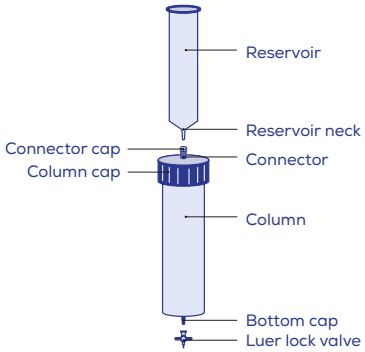
OPERATIONAL RECOMMENDATIONS

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. Ensure the sample buffer is the same temperature as the column (preferably 18–24 °C).
4. **Only use freshly filtered (0.22 µm) and degassed buffer to avoid contamination or bubbles in the resin.**

OPERATING INSTRUCTIONS

qEV100 GEN 2 COLUMN SPECIFICATIONS

Sample Load Volume	100 mL
Column Volume	600 mL
Buffer Volume	165 mL (70 nm) 185 mL (35 nm)
Optimal Fraction Size	50 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 column temperature is within this range.**
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 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.

COLUMN FLUSHING

1. Open the luer lock valve (handle is vertical) and allow the 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.

SAMPLE COLLECTION

1. Filter or centrifuge the biological sample to remove large particulate matter. Refer to operational recommendations above.
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 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[1]. 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)[2]. Refer to Figure 1.
8. Use the valve to pause the flow between collected volumes.

COLUMN CLEANING AND STORAGE

1. After the desired volume has been collected, flush the column with 1200 mL of buffer, followed by 200 mL of 0.5 M sodium hydroxide (NaOH), followed by another 1200 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. Close the valve on the bottom of 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. 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.

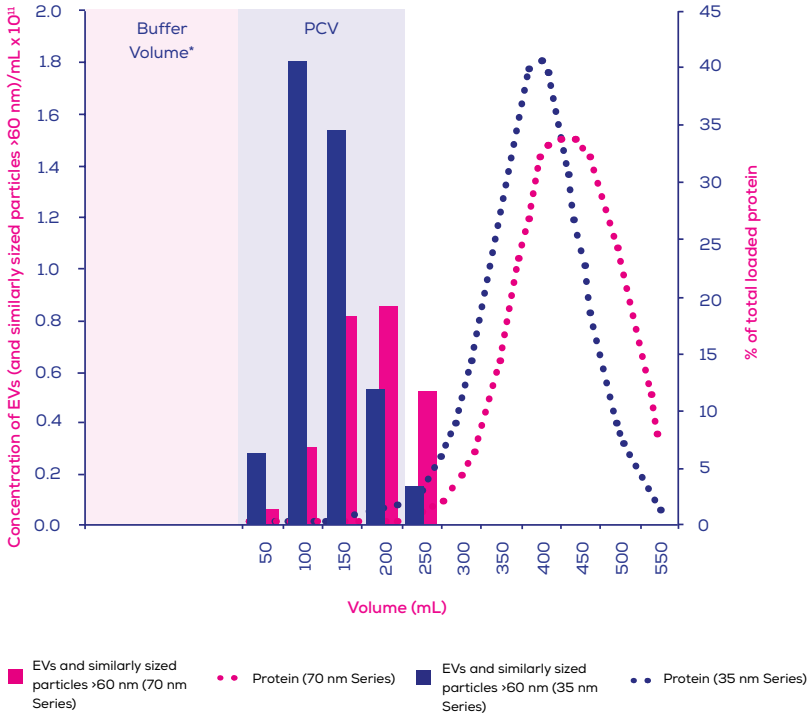


Figure 1. Comparison of total protein elution levels and concentration of extracellular vesicles (EVs) and similarly sized particles >60 nm between qEV100/35 nm Gen 2 and qEV100/70 nm Gen 2 columns with 100 mL of human plasma loaded, normalised for buffer volume. Particle concentration was measured using an Exoid and protein levels by bicinchoninic acid (BCA) assay.

*Nb: buffer volumes for human plasma samples differ for qEV100/35 nm Gen 2 (185 mL) and qEV100/70 nm Gen 2 (165 mL).

[1] Buffer volume: 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.