QUICK START GUIDE

FOR qEV100 GEN 2 COLUMNS (20 nm, 35 nm & 70 nm)

This quick start guide provides general operating instructions. For more detailed information, see the qEV100 user manual and other resources at support.izon.com

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

The qEV column contains < 0.1% ProClin 200 or < 0.1% sodium azide*, both of which are harmful if swallowed or in contact with skin. Refer to the user manual for more information.

*Izon is transitioning from the use of sodium azide to ProClin 200 for the storage of qEV columns. For information on how to identify which storage buffer is in your column, visit support.izon.com

STORAGE BEFORE USE

Store unused qEV columns upright at room temperature.

INTENDED USE

qEV columns are intended for use by professional personnel only.

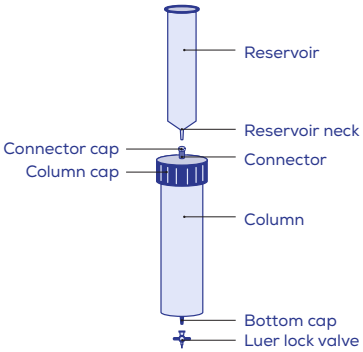
OPERATIONAL RECOMMENDATIONS

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 COLUMN SPECIFICATIONS

Sample Load Volume*	Up to 100 mL
Recommended Buffer Volume**	210 mL (20 nm) 210 mL (35 nm) 190 mL (70 nm)
Recommended PCV**	100 mL
Optimal Fraction Size	25 mL
Column Volume	600 mL



*Larger volume columns, like the qEV100, are more affected by sample-dependent properties like EV and protein load, meaning that your optimal loading volume might be less than 100 mL. We suggest testing various loading volumes of your sample to identify when the relationship between the input volume and the output EV concentration is no longer linear. After this point, the column will be overloaded and there will be a diminishing return of EVs and decreased isolate purity.

**For information on selecting Buffer Volume and Purified Collection Volume values, refer to the user manual.

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 minimises potential effects of storage buffer 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.

> ⚠ Flushing the column with a large volume of buffer after running your sample is not sufficient to clean the column completely and may result in carry-over from previous samples.

2. If storing for future use, store in PBS containing a bactericide or bacteriostatic agent (e.g., 0.05% ProClin 200 or 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. Recap the column and rinse the external surface of the column, including the RFID tag, with water and thoroughly dry before storing in an upright position.
4. Columns containing a bacteriocide or bacteriostatic agent can be stored upright 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.

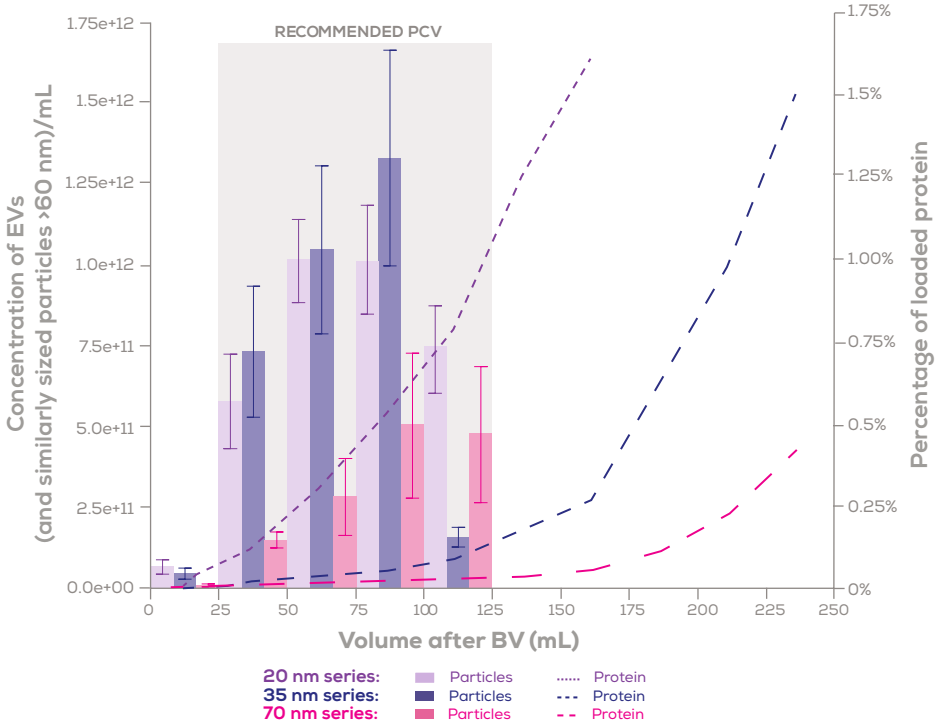


Figure 1: Elution profiles of qEV100 columns (20 nm, 35 nm, and 70 nm) with 100 mL of human plasma loaded. Particle concentration was measured using the Exoid, and protein concentration was measured using a bicinchoninic acid (BCA) assay. Particle concentration is expressed as the mean ± standard error, while the percentage of protein recovered is depicted using the mean value. n=3 for each column series. BV = Buffer Volume[1]; PCV = Purified Collection Volume.[2]

[1]Buffer Volume (BV): The BV is defined by the Purified Collection Volume (PCV); it is the volume that elutes before the PCV, and therefore contains very few EVs. The BV may differ by resin type.
[2]Purified Collection Volume (PCV): A customisable, collected volume containing purified particles of interest. The PCV can be adjusted to suit different priorities, e.g., to maximise EV recovery, purity, or concentration.