QUICK START GUIDE

FOR qEV1 COLUMNS
(20 nm, 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% 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 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 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). 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

qEV1 COLUMN SPECIFICATIONS

Sample Load Volume	1 .0 mL
Column Volume	13.5 mL
Buffer Volume*	4.0 mL (35 nm & 70 nm) 3.8 mL (20 nm)
Optimal Fraction Size	0.7 mL

*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 operational temperature range is reached.**
2. Carefully remove the top cap only.
3. Attach the column in an upright position to a stand ready for use. Automatic Fraction Collectors (AFCs) and qEV Racks are available from store.izon.com
4. Attach a column reservoir if available, and top up with buffer.

COLUMN FLUSHING

1. Remove the bottom cap 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. 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 Figure 1.

COLUMN CLEANING AND STORAGE

1. After the desired volumes have been collected, flush the column with 13.5 mL of 0.5 M sodium hydroxide (NaOH) followed by 27 mL of buffer before loading another sample..
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. 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.

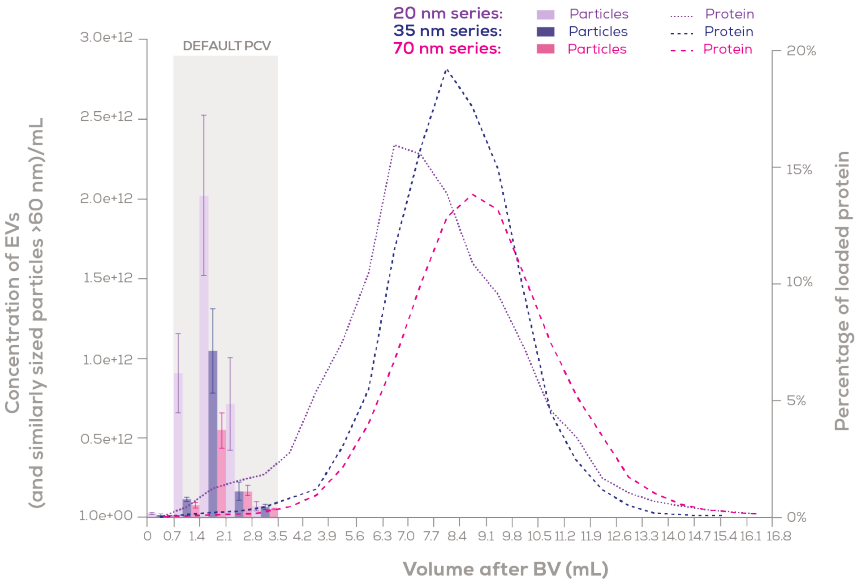

Figure 1: Elution profiles of qEV1 columns (20 nm, 35 nm, and 70 nm) with 1 mL of human plasma loaded. Particle concentration was measured using the Exoid, and protein concentration was measured using a bicinchoninic acid (BCA) assay. Particle concentration is expressed as the mean ± standard error, while the percentage of protein recovered is depicted using the mean value. n=3 for 20 nm and 35 nm, n=6 for 70 nm. 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. The PCV programmed on the AFC is referred to as the default PCV.