

QUICK START GUIDE

FOR qEV2 GEN 2 COLUMNS
(20 nm, 35 nm & 70 nm)



This quick start guide provides general operating instructions. For more detailed information, see the qEV2 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 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.

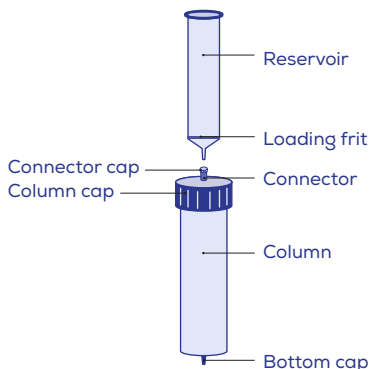
OPERATIONAL RECOMMENDATIONS

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) 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. Use freshly filtered (0,22 µm) and degassed buffer.

OPERATING INSTRUCTIONS

qEV2 GEN 2 COLUMN SPECIFICATIONS

Sample Load Volume	2 mL
Column Volume	45 mL
Buffer Volume*	14.6 mL (20 nm) 15.9 mL (35 nm) 15.2 mL (70 nm)
Optimal Fraction Size	2 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 column temperature is within this range.
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 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. Continue to allow buffer to run through the column until it stops at the loading frit.
3. 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.

4. Immediately start collecting the buffer volume¹. The buffer volume includes the volume displaced by loading the sample.
5. Allow the sample to run into the column. The column will stop flowing when all of the sample has entered the loading frit.
6. Top up the reservoir 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.

7. 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 90 mL of buffer, followed by 4 mL of 0.5 M sodium hydroxide (NaOH), followed by another 90 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 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. Place the bottom cap on 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. 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.

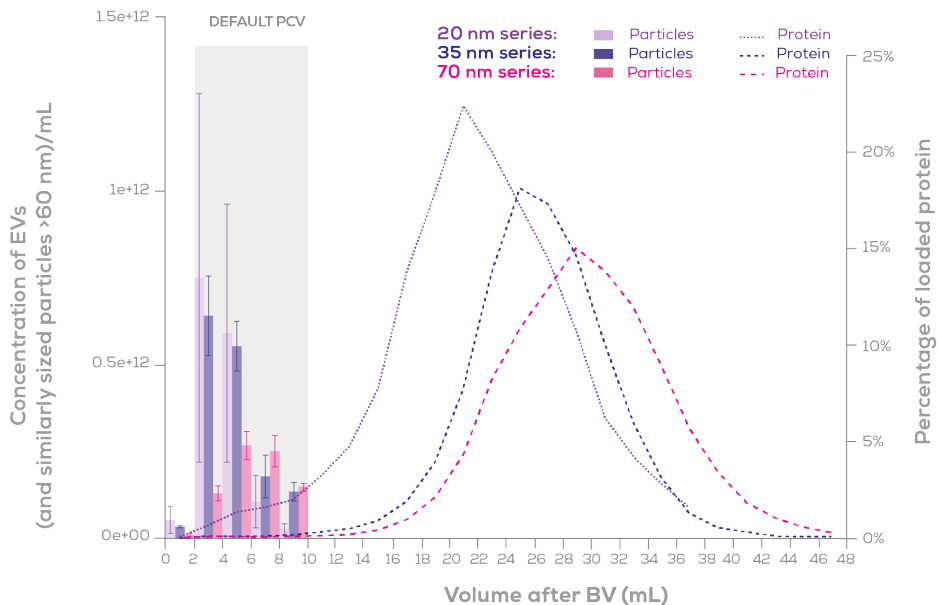


Figure 1. Elution profiles of qEV2 columns (20 nm, 35 nm, and 70 nm) with 2 mL of human plasma loaded. Particle concentration was measured using the Exoid, and protein concentration was measured using a bicinchoninic acid (BCA) assay. Particle concentration is expressed as the mean \pm standard error, while the percentage of protein recovered is depicted using the mean value. $n=3$ for 20 nm, $n=6$ for 35 nm and 70 nm. BV = Buffer Volume¹; PCV = Purified Collection Volume².

¹Buffer 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.

²Purified 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.