QUICK START GUIDE

FOR qEVORIGINAL GEN 2 SMART COLUMNS (35nm & 70nm)

This quick start guide only provides general operating instructions. For more detailed information, you can download the full library of qEV User Manuals and Technical Notes from the Izon support portal at support.izon.com

Safety Data Sheets are available at www.izon.com/resources

INTENDED USE

Izon qEV SMART columns isolate extracellular vesicles from biological samples. The qEV SMART columns are equipped with RFID chips for use with the Izon Automatic Fraction Collector (AFC). These chips will not impact manual use. The qEV column is intended to be used in research laboratories by professional personnel for research use only. The qEV column is 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 xg for 10 minutes to remove any cells and large particles. Re-centrifuge the supernatant at 10,000 xg for 10 minutes. For microvesicle isolation, use lower g-forces for the second centrifugation step.
2. For larger volume samples, it is possible to concentrate the sample before loading onto the qEV column. This is not applicable for serum and plasma samples that have very high levels of protein. Izon recommends using Merck Millipore concentration devices, Amicon® Ultra Centrifugal filters.
3. Izon recommends single use of columns if you intend to analyse vesicles for nucleic acids.
4. Ensure that 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

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. qEV Racks and Automatic Fraction Collectors are available from Izon Science.
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 one column volume 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.
3. Immediately start collecting the buffer volume[1] (this includes the sample volume).
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.
6. Once the buffer volume is collected, continue to collect the Purified Collection Volume (PCV)[2]. Refer to Figures 1 and 2.
7. 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. **Avoid stopping the column flow during the run for long periods of time to ensure accurate EV separation.**

COLUMN FLUSH AND STORAGE

1. After the desired volumes have been collected, flush the column with 8.5 mL of 0.5 M Sodium Hydroxide (NaOH) followed by 17 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).
3. Store the column at +4 to +8 °C.

qEVORIGINAL GEN 2 COLUMN SPECIFICATIONS

Sample Load Volume (mL)	≤ 0.5
Column Volume (mL)	8.5
Buffer Volume (mL)	2.5
Optimal Fraction Size (mL)	0.4
Buffer required per sample collection (mL)	5.0

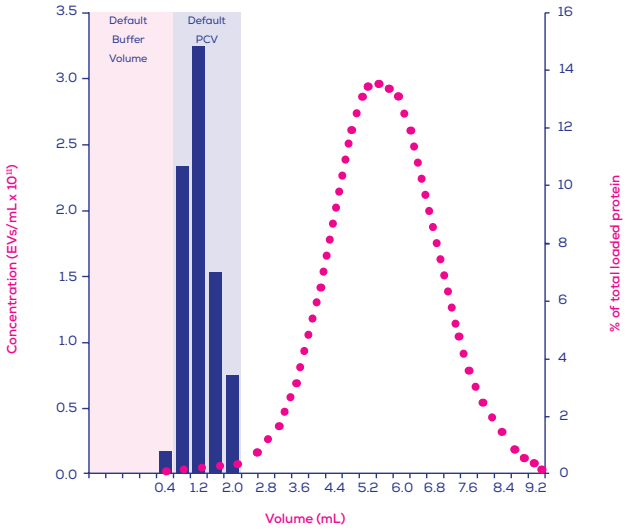

Figure 1. Typical elution profile for a qEVoriginal/35nm Gen 2 column with 0.5 mL of human plasma loaded; proteins elute in a later volume than vesicles. The vesicle concentration was measured using an Exoid and protein levels by BCA analysis.

- EVs and similarly sized particles
- Protein absorbance

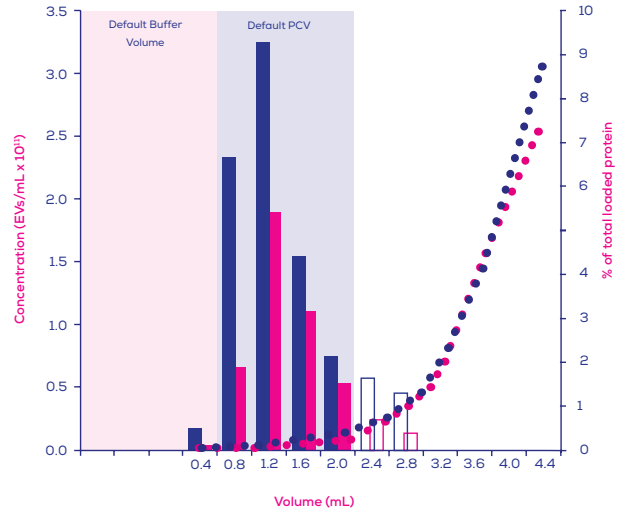

Figure 2. Comparison of protein and EV elution profiles between a qEVoriginal/35nm Gen 2 and a qEVoriginal/70nm Gen 2. Outlined columns are theoretical values based off pooled sample measurements.

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

[1] Buffer volume - 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. Refer to the full user manual for information regarding choosing an appropriate PCV.