

QUICK START GUIDE



FOR qEVORIGINAL 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

INTENDED USE

qEV columns 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 to be used 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. This is not applicable for serum and plasma samples, which 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 on analysing vesicles for nucleic acids.
4. Ensure 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

qEVORIGINAL GEN 2 COLUMN SPECIFICATIONS

Sample Load Volume	≤ 0.5 mL
Column Volume	8.5 mL
Buffer Volume	2.5 mL
Optimal Fraction Size	0.4 mL
Buffer required per sample collection	5.0 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 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 your downstream applications are affected by sodium azide, flush with at least 2 column volumes 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 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 volume¹ (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 Figures 1 and 2.

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 either a buffer containing a bacteriostatic agent (e.g. 0.05 % w/v sodium azide), or 20% ethanol.
3. Columns 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.

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.

