

# QUICK START GUIDE

FOR qEVSINGLE LEGACY 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](https://support.izon.com)

Safety Data Sheets are available at [support.izon.com/safety-data-sheets](https://support.izon.com/safety-data-sheets)



The qEV column contains < 0.1% sodium azide, which is potentially fatal if swallowed or in contact with skin. Please refer to the user manual for more information.

## 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 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. Prior to loading the column, centrifuge samples 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. qEVsingle columns are recommended for single use only.
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

### qEVSINGLE COLUMN SPECIFICATIONS

Sample Load Volume		0.15 mL
Column Volume		3.5 mL
Buffer Volume	qEV/35	0.8 mL
	qEV/70	1.0 mL
Optimal Fraction Size		0.2 mL
Buffer required per sample collection		2.0 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. Automatic Fraction Collectors (AFCs) and qEV Racks are available from [store.izon.com](https://store.izon.com)
4. Top up column 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 will also minimise potential effects of sodium azide 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.



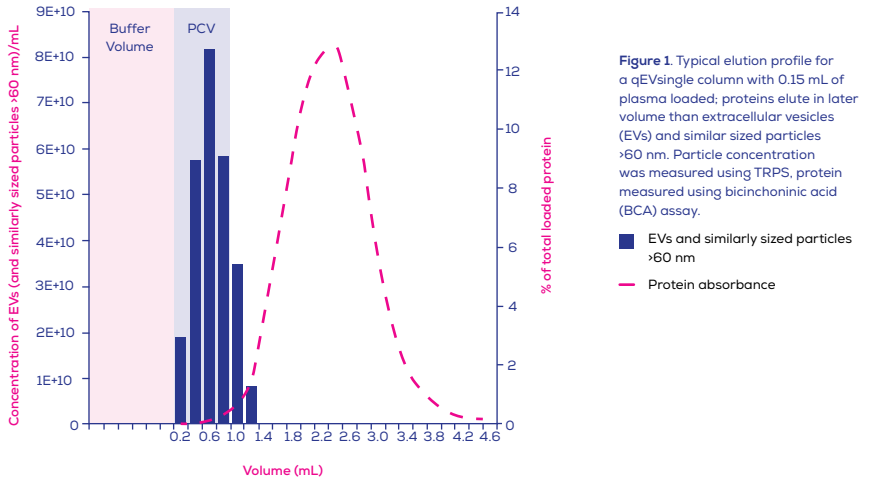
Avoid stopping the column flow during the run for long periods of time to ensure accurate EV separation.

3. Immediately start collecting the buffer volume<sup>1</sup> (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 column with buffer and continue to collect the buffer volume.



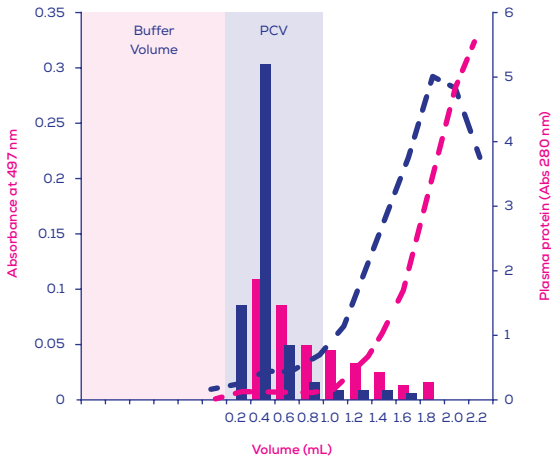
To collect accurate volumes, only load the required volume to the top of the column, wait for the volume to run through until the flow stops and repeat.

6. Once the buffer volume is collected, continue to collect the Purified Collection Volume (PCV)<sup>2</sup>. Refer to [Figures 1 and 2](#).



**Figure 1.** Typical elution profile for a qEVsingle column with 0.15 mL of plasma loaded; proteins elute in later volume than extracellular vesicles (EVs) and similar sized particles >60 nm. Particle concentration was measured using TRPS, protein measured using bicinchoninic acid (BCA) assay.

■ EVs and similarly sized particles >60 nm  
 - Protein absorbance



**Figure 2.** Comparison of total protein elution levels and recovery of 69 nm liposomes between a qEVsingle/35 nm and a qEVsingle/70 nm column.

■ qEV/35 particles  
 - qEV/35 protein  
 ■ qEV/70 particles  
 - qEV/70 protein

<sup>1</sup> Buffer volume (BV): The volume of liquid that corresponds to the volume before the Purified Collection Volume (PCV). This volume may be different for different resin types in the same column size series.

<sup>2</sup> Purified Collection Volume (PCV): The volume immediately succeeding the Buffer Volume, containing particles of interest purified from the loaded sample. The PCV can be customised to accommodate different preferences, e.g., to maximise the recovery of extracellular vesicles, or to maximise protein removal.