QUICK START GUIDE

FOR qEVSINGLE (35nm & 70nm) SMART COLUMNS

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.

Safety Data Sheets are available at izon.com/sds

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 x g for 10 minutes to remove any cells and large particles. Re-centrifuge the supernatant at 10,000 x g 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. qEVsingle columns are recommended for single use only.
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. 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 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.
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 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 & 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.**

qEVSINGLE COLUMN SPECIFICATIONS

Sample Load Volume		≤ 0.150 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

[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.

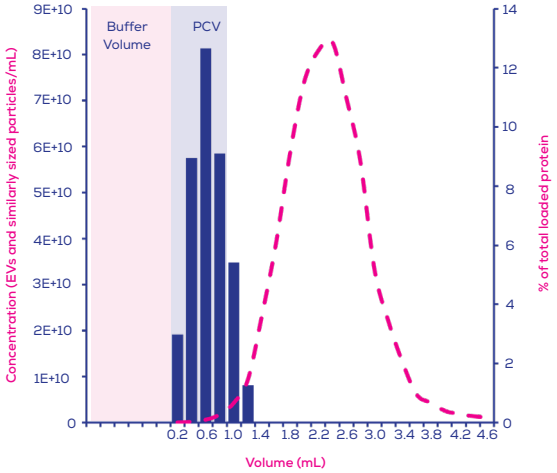

Figure 1. Typical elution profile for a qEVsingle column with 150 µL of plasma loaded; proteins elute in later volume than vesicles. Particles measured using TRPS, protein measured using BCA assay.

EVs and similarly sized particles

Protein absorbance

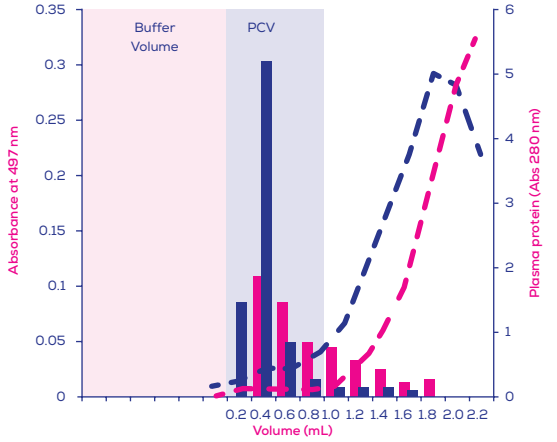

Figure 2. Comparison of total protein elution levels and recovery of 69 nm liposomes between a qEVsingle/35nm and a qEVsingle/70nm.

qEV/35 particles

qEV/35 protein

qEV/70 particles

qEV/70 protein