Recommended qEV size and sample preparation protocol by sample type:

Sample type	Sample characteristics	Sample volume	Suitable qEV column type	Sample preparation protocol
Cell culture and urine	High-volume, Low protein, Low EV content	150-1000 mL	qEV10	- Spin samples to remove cells, debris and large EVs - Pre-concentrate samples down to 10 mL - Run on qEV10 (flush and re-use column if needed) - Concentrate pooled EV fractions if necessary
Plasma and serum	Varied volume, High protein, High EV content	5-50 mL	qEV10 or qEV2	- Spin samples to remove cells, debris and large EVs - Run on qEV10 or qEV2 - Concentrate pooled EV fractions if necessary (high-protein samples should not be pre-concentrated)
Low-viscosity biological fluids	Low volume, Varied EV and protein content	0.1-0.5 mL	qEVoriginal or qEVsingle	- Spin samples to remove cells, debris and large EVs - Run on qEVoriginal or qEVsingle - Concentrate pooled fractions if necessary
Viscous biological fluids	Low volume, Varied EV and protein content	0.1-0.5 mL	qEVoriginal or qEVsingle	- Suspend samples in PBS to reduce viscosity - Spin samples to remove cells, debris and large EVs - Run on qEVoriginal or qEVsingle - Concentrate pooled fractions if necessary

Recommended qEV gel type for specific downstream analysis requirements:

Gel type:	Specifications:	Advantages:	Limitations:
70 nm	Best for isolating particles with a size range of 70-1000 nm.	- The 70 nm size cut-off allows for higher-purity EV fractions in terms of protein and lipoprotein content.	- The 70 nm size cut-off may also exclude smaller EV size populations. - The EV fractions are less concentrated and the overall yield is lower using this gel.
35 nm	Best for isolating particles with a size range of 35-350 nm.	- The overall EV yield is 2-3 times higher using this gel than the 70 nm gel.	- The sample size distribution will be biased towards the smaller EVs, as the yield for larger EVs over 350 nm is not as high as with the 70 nm gel. - Large proteins and lipoproteins will also elute in the EV fractions, potentially compromising the purity of the EV fractions.

To summarise the table above, if you are interested specifically in exosomes (size: 35-150 nm) or outer-membrane vesicles (size: 50-200 nm), and your downstream analysis techniques will not be affected by the protein or lipoprotein content of the samples, then use the 35 nm gel. For all other applications, use the 70 nm gel option, or modify your sample preparation technique to remove contaminants before using the 35 nm gel.

Sample cleaning methods for different target EV populations

The primary function of qEV columns is to gently and quickly remove small contaminant particles like free proteins and small lipoproteins from EV samples, as these contaminants are retained by the small pores in the resin beads, and elute in later fractions than EVs. However, the columns are not designed to separate large particles in the sample (such as cells, cell debris and very large vesicles) from EVs. These large contaminants may elute in the EV fractions if present in the sample.

In order to prepare high purity EV samples, these large contaminants should be removed by using a bench centrifuge before loading the sample onto a qEV. This cleaning step should also be done prior to any sample concentration steps, as the rupture of cells against concentration membranes may cause the release of cell debris and other types of vesicles into the sample,

Use the following table as a guide to clean samples before using a qEV:

Target EV population	First spin	Second spin	Final cleaning steps for TRPS analysis
All EVs 30-1000 nm	Spin all samples at 1500 g for 15-30 minutes to remove cells. Discard pellet.	Spin all samples at 10,000 g for 10 minutes to remove cell debris. Discard pellet.	Supernatant should either be snap frozen at -80 °C, or filtered with a 1µm syringe filter and loaded onto a qEV column immediately.
Microvesicles 100-600 nm			Supernatant should either be snap frozen at -80 °C, or filtered with a 0.8 µm syringe filter and loaded onto a qEV column immediately.
Exosomes and OMVs 30-200 nm			Supernatant should either be snap frozen at -80 °C, or filtered with a 0.22 µm syringe filter and loaded onto a qEV column immediately.

Additionally, if working with whole blood, leave to sit at room temperature for 30 minutes, and centrifuge at 1200 g for 10 minutes at 4 °C to separate plasma. Spin again at 1800 g for 10 minutes at 4 °C to remove remaining platelets (and snap-freeze aliquots at -80 °C for storage if required). If working with cell culture, spin at 300 g for 10 minutes at 4 °C to remove cells before any other steps.

Commonly-used sample concentration protocols and filter devices

Dilute biological samples with low protein content, such as urine and cell culture media, should be concentrated before qEV isolation, to enable larger volumes to be processed as well as increase the EV yield. High-protein biofluids such as plasma or serum should be concentrated only after the protein has been separated from the sample via qEV size exclusion chromatography.

These are the types of filters that can be used to concentrate biological samples:

Maximum sample volume (mL)	Minimum concentrated volume (mL)	Recommended concentration device	Centrifuge requirements
0.5	0.05	Merck Microcon-30kDa Centrifugal Filter	Standard lab microfuge, up to 14,000 g
15	0.5	100 kDa cut-off Amicon Ultra 15	Low speed bench centrifuge, up to 4000 g
70	0.35	100kDa cut-off Centricon Plus-70	Swinging bucket bench centrifuge, up to 10,000 g
>150	For rapid concentration of high-volume samples, use pressure-based concentration devices such as Amicon Stirred cells units, or ultracentrifuge-based Pellicon Tangential Flow devices.		

Generic sample concentration protocols may require modification depending on the sample used and other preparation steps needed prior to qEV use. For example, urine samples that have been pre-concentrated should also be centrifuged again at 10,000 g for 10min prior to loading onto a qEV column, to exclude any precipitates that may have formed during the concentration process.

For Amicon 100 kDa cut-off filters, particles or proteins which are <100 kDa will pass through the filter and be removed from the sample, along with excess water during the concentration process. This will exclude common contaminating proteins like bovine serum albumin (a 66 kDa protein 5 nm in diameter) from your samples.

If you are isolating exosomes from cell culture media, and have been recommended the 35 nm qEV resin type, but want to prevent larger contaminating proteins like BSA eluting in the EV fractions, you could perform the concentration step detailed above twice (by re-diluting the sample into PBS after the first concentration spin, and repeating the concentration step again) as an extra protein wash.