

# Single-use size-exclusion chromatography columns for analytical scale Extracellular Vesicle isolation

Izon Science's qEVsingle Size Exclusion Columns enable the rapid isolation of extracellular vesicles (EVs) from 100µL of complex biological fluids or concentrated cell culture supernatants. Designed for analytical-scale samples, each qEVsingle column efficiently removes background proteins, lipids, solutes, cell debris, and other particulates to improve the sensitivity and accuracy of downstream assays (e.g., TRPS, protein profiling, RNA profiling). Vesicles are isolated in 15 minutes allowing large-scale EV sample studies to take place in a short period of time compared to ultracentrifugation-based isolation methods. Their single-use design ensures sterile (no cross-contamination) operation. qEVsingle columns are built to ISO 13485 certified quality standards, which ensures consistency of operation and is a requirement for clinical use. The gEVsingle columns complement the existing standard gEV SEC columns, which are specified for larger sample volumes (500µL) and multiple use (up to 5 times). Both products are available to purchase directly from Izon Science (store.izon. com) or your local approved distibutor. gEVsingle columns ship in boxes of 20.

# **Key Features**

- Analytical-scale sample volume (100µL)
- Designed for large-scale clinical studies
- Reliable EV isolation in minutes
- Optimised for exosomal RNA analysis
- Compatible with qEV Rotary Holder for higher throughput
- Manufactured to ISO 13485 quality standards



## Isolation of Exosomes with qEVsingle SEC Columns

Size Exclusion Chromatography (SEC) is the most effective method for isolating exosomes from cell culture supernatants and complex biological fluids. Since the separation is based on size (Figure 1), vesicles flow through the column un-retained and elute in the void volume.

Proteins and other contaminants that are smaller than the pores of the stationary phase are retained by the column and elute later. Other methods that are non-specific in nature require overnight incubation of vesicles with precipitation buffer. As a result, vesicular and non-vesicular particles are isolated together so additional steps are needed to separate EVs from contaminating particles. In contrast, isolations using qEVsingle SEC columns take 15 minutes , remove >99% of contaminating background proteins and up to 95% of high-density lipoprotein (HDL) contaminates in a single isolation. SEC columns, built to quality standards, provide the only separation method that can be standardized sufficiently for medical trials and subsequent clinical use.



SEC works by trapping smaller molecules in the pores of the stationary phase. The larger molecules or particles flow past the pores because they are too large to enter. Larger molecules or particles flow through the column faster than smaller molecules and elute first. Smaller molecules have longer retention times and elute later.

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## **Reproducible, Efficient and Fast Isolation**

qEVsingle columns are designed for single-use, removing the need for a regeneration step and ensuring the highest purity and consistency. They are gentle on vesicles. As opposed to ultracentrifugation and precipitation, SEC purified EVs retain their biological function and structure. SEC purified exosomes are free of contaminating proteins.

Vesicle recovery from column-to-column is very reproducible. Since samples are isolated without extreme centrifugal force, there are no protein aggregates or vesicle aggregation. In comparison, vesicle preparations using ultracentrifugation show variable recovery and require 2 to 96 hours to complete. The highly viscous and hyperosmotic sucrose gradients, used in UC affect the biological function of your vesicles post isolation. SEC purified vesicles are isolated using phosphate-buffered saline solution (PBS) which maintains the essential biological structure of the EVs.



#### Figure 2

qEVsingle elution profile

Typical qEVsingle Size Exclusion Column elution profile from a  $100\mu$ L plasma sample. EVs (blue) elute in fractions 6 to 9, while bulk free proteins (red) elute in later fractions.

#### **Single Use**

The small size and price makes these columns ideal for single use applications where sample cross contamination needs to be avoided, e.g. DNA, RNA or patient sample analysis.

For repetitive, limited volume applications these smaller columns provide the ideal solution, resulting in reduced sample dilution for small samples during separation, compared with the larger standard qEV columns.

### Simple Method to Isolate Extracellular Vesicles

Isolating EVs using qEVsingle Size Exclusion Columns takes only 15 minutes. The simple five-step procedure is:

## Procedure

- 1. Rinse the column with PBS.
- 2. Pipette 100 µL of sample onto the column.
- 3. Add PBS to the column to elute EVs.
- 4. Collect fractions.

Figure 2 shows the analysis of fractions collected from a plasma sample. A qNano Gold System, with Tunable Resistance Pulse Sensing, reliably measured the presence of EVs in a quantitative fashion. Relative protein concentration was measured by absorbance at 280 nm. The data shows that fractions 6 to 8 have the highest concentration of EVs and the contaminating plasma proteins elute in fractions 10 to 23. Depending on the sample, fractions 6 to 8 or fractions 6 to 10 ( $200\mu$ L each) are pooled if downstream assays require higher EV purity or higher EV yield, respectively.

### Focus on Your Research, Not Your Vesicle Isolation

Characterization of EVs first requires their isolation from cell culture supernatants or complex biological fluids. Numerous protocols and reagents exist to isolate EVs, but their results vary greatly. qEVsingle SEC columns are the fastest, easiest and most effective method to isolate EVs. Unlike other methods, qEVsingle columns are gentle on your EVs so they retain their biological properties and are free of contaminating proteins, vesicle aggregates and HDL. qEVsingle SEC columns are convenient, available off-the-shelf and quality assured so you can spend your time focusing

#### **Ordering Information**

Website:	store.izon.com
Email:	orders@izon.com
Part number:	SP2
Description:	qEVsingle (20 columns/package)



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