Version 0.01

qEV Size Exclusion Column

Beta 1.0 – Specifications and operational guide

Simple Quick Purification of Extracellular Vesicles

Important. Measurements and specifications in this guide relate to beta-test results and should be taken as indicative of performance. Specifications are subject to change

Features

Izon qEV columns purify biological samples to obtain the extracellular vesicles.

Samples[*] suitable for qEV use

- Blood derived samples, such as Serum, Plasma
- Saliva
- Urine
- Cell culture media

The advantages gained using an Izon qEV column[i] include;

- Approximately 20 minute separation time.
- Greatly reduced risk of protein complex formation and vesicle aggregation (as can occur in ultracentrifugation and other commercial exosome precipitation methods
- Buffers with physiological osmolarity and viscosity can be used.
- A gentle rapid method for maximizing recovery of biological function
- Vesicle recovery is expected to be 50% or greater
- Protein Removal Ratio > 1000 [†]

[*] Note some 'raw' samples cannot be directly run on qEV without preparation steps, such as two or more stage of centrifugation to prepare the sample (e.g. blood, Saliva). See Izon support website for example protocols

[†] This reflects the ratio of protein in sample to that eluted in vesicle peak fractions, based on independent testing of 7 columns, can be as high as 11000x.

- HDL purification > 8 fold

Specifications

Bed Volume	10ml
Sample volume	1ml
Void volume	3.5ml ±0.25ml
Separation Size	70nm nominal
Largest size passable	1um
Frit pore size	20um
Buffer	PBS buffer (anti-bacterial <0.05% sodium-azide)
Flow-rate	Typically 0.8 to 1.2 ml/min
pH Stability Working Range	3 - 13
pH Stability Cleaning-in-Place (CIP)	2 – 14
Shelf Life	>18mths (if stored correctly).
Life after first use	Dependent on use and storage

Operation

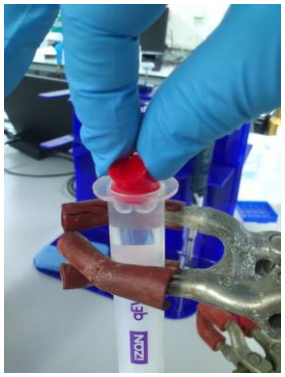
Safety precautions

Always use appropriate personal protection devices such as lab coats, gloves and safety glasses when handling qEV columns.

- The column anti-bacterial solution contain 0.05% w/v sodium azide. Sodium Azide in larger quantities is toxic so direct contact with skin or eyes should be avoided.
- Waste buffer should be disposed of in a safe manner. Sodium azide accumulation over time in copper pipes can result in explosion.
- Biological samples can be hazardous, consult you laboratory safety officer for information on safe handling of your sample when using the qEV column

Storage

Store the column at +4 to +8 °C in the presence of a bacteriostatic agent, e.g. 20% ethanol, < 0.05% w/v sodium azide.



Preparation for use

- Place the column in a holder and level it (make sure the column is vertical).
- Leave the bottom luer-slip cap in place.
- Remove the top-cap carefully and slowly. Pinch it tightly so as to break seal and reduce vacuum from removal

Column equilibration

- Remove the luer cap.
- Rinse the column with at least 10 mL PBS buffer. Note the time for 5ml of buffer to flow through in your lab book – this is useful for detecting when to clean the column.

- *Do not allow the column to run dry (top frit must stay wet). If run dry the column may not function correctly*
- *Use only freshly made filtered (0.2um) buffer to avoid introducing particulate contamination*

Sample Application

Some samples may elute fractions earlier or later. For the most exact results collect and measure all fractions from 3ml to 4ml to determine where the peak fractions elute

1. With the lower luer cap on. Pipette out the buffer above the top-frit
2. Pipette the sample in.
3. When ready to begin collecting fractions remove the lower luer cap.
4. Immediately start collecting 3.5ml (void volume)
5. Add more buffer as the sample enters the column, but add no more than 2 mL above the top frit.
6. Immediately after the void volume collect the vesicle fraction of 1ml. For minimizing protein contamination collect first 0.5ml only.
7. After collection of the vesicles, flush the column with at least 10ml of buffer and store as indicated above. Note the time for 5ml of buffer to flow through in your lab book – this is useful for detecting when to clean the column.

Re-use

How to detect when the column is compromised and needs cleaning.

- Flow rate begins to slow over original flow rate. It is always recommended to time the rate for the void volume and vesicle fraction and note these in your lab book.
- Recovery rate drops significantly. It is difficult to measure the recovery rate accurately as the before concentration of most biological samples will not be possible to measure easily. It can be inferred from the expected vesicles recovered reduce with subsequent operation. If this is the case, run Izon calibration particles through your column (in PBS buffer), do at least 2000 counts before and after, and the recovery rate should be >90% ± 20%
- Colour change at the top of the column
- A space between the upper adaptor and the gel surface

Regeneration

Regeneration is normally performed by washing with 2‑3 column volumes of buffer, followed by re-equilibration in the new buffer (if changing conditions).

In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed using the cleaning procedure described below.

Cleaning

Remove precipitated proteins, non-specifically bound proteins and lipoproteins by washing the column with one column volume of 0.5 M NaOH, then flush the column with at least 3-5 volumes of PBS and check the pH of the elution media with litmus paper.

Remove strongly non-specifically bound proteins, lipoproteins and lipids by washing the column with two column volumes of a non-ionic detergent solution, e.g. 0.1% Triton TM X-100, followed by at least 2–3 column volumes of eluent buffer.

The cleaning procedures given above can also be performed with the gel on a Buchner funnel.

Sanitization

Sanitization reduces microbial contamination of the gel to a minimum. Wash the column with 0.5 M NaOH. Re-equilibrate the column with 3‑5 bed volumes of sterile buffer and check the pH of the elution media with litmus paper.

Notes

- Degassed buffers will help eliminate air bubbles forming in the gel bed.
- It is recommended to use a buffer with an ionic strength of 0.15 M or greater to avoid any unwanted ionic interactions between the solute molecule and the matrix.
- To avoid clogging of column filters, it is recommended to filter or centrifuge the biological sample to get rid of particulate matter.

Separation Ability

Peak Vesicles

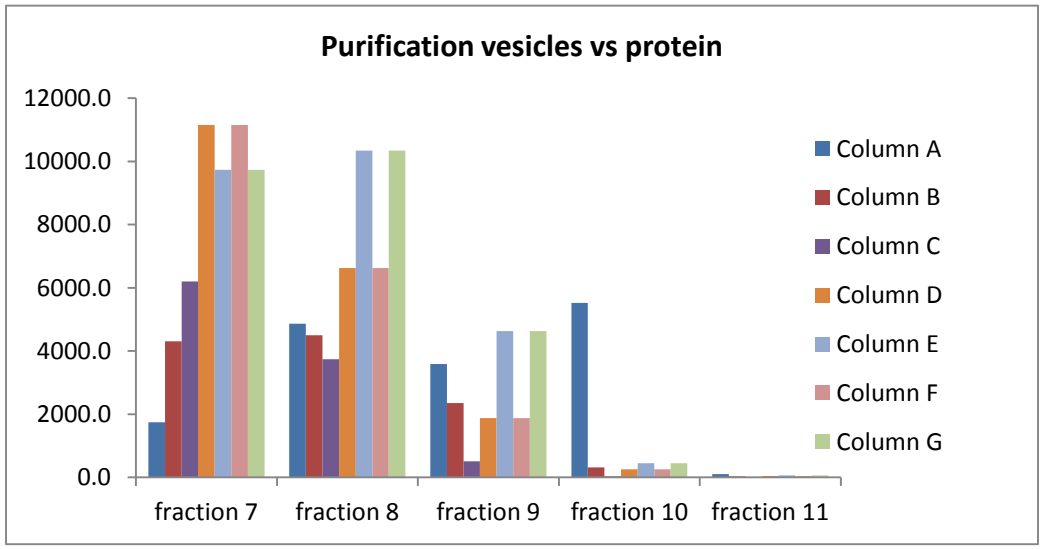
- Peak vesicles elution occurs at 3.75ml ± 0.25ml.
- Recovery rate in the peak fractions is 75% ± 30%.
- For 0.5ml fraction, the peak fractions occur in fractions 7 and 8. If higher purification is desired, only collect fraction 7.

Purification

The following figure shows the indicative increase in ratio of vesicles / protein is improved in each fraction, that is,

$$Purification = \frac{Start\ Vesicles\ per\ ml\ /\ conc.protein\ ug/ml}{Collected\ Vesicles\ per\ ml\ /\ collected\ conc.protein\ ug/ml}$$

The vesicles were identified using an antibody for CD61, Protein concentration was identified using a Bradford protein assay.



Protocols for Preparation from Common EV sources

Biological Samples vary considerably and it is impossible to provide a comprehensive list of protocol specific to each sample; if you are unsure of what to do to prepare your sample, please contact support@izon.com for assistance.

Glossary

Chromatography	A method, used primarily for separation of the components of a sample. The components are distributed between two phases, one is stationary while the other one moves. The stationary phase is either a solid, a solid supported liquid, or a gel. The stationary phase may be packed in a column, spread as a layer or distributed as a film. The mobile phase may be gaseous or liquid.
Bed volume	Volume of packed material and void volume
Fraction	Indicates a particular volume collected from column, specified numerically for a given size. That is fraction 7 of 0.5ml fractions refers to the 0.5ml volume collected after 3.0ml and up to 3.5ml
Degassing	degassing involves subjecting a solution to vacuum to "boil" off excess dissolved air e.g. applying a vacuum to a flask, that`s how we are doing it. If a vacuum based system is not available, you could try to filter your buffer through 0.22 um filter to remove bubbles
Flow Rate	The volumetric flow in mL/min of the carrier liquid
Agarose	High molecular weight polysaccharide used as a separation medium in bio-chromatography. It is used in bead form, often in gel-filtration chromatography, with aqueous mobile phases.
Void time	The elution time of an un-retained peak; also called the dead time and the holdup time. The void volume is determined by multiplying the void time and the flow rate.
Void volume	The total volume of mobile phase in the column; the remainder of the column is taken up by packed gel material. It denotes the excluded volume in SEC.
Vesicle fraction	The fraction that the vesicles appear in.
Recovery rate	The percentage of vesicles that come out of the column compared with what went in.

Enquire at info@izon.com and ask how we can improve the quality of your particle analysis research.

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[i] A.N Boing et-al; "Single-step isolation of extracellular vesicles by size-exclusion chromatography", Journal of Extracellular Vesicles 2014, 3: 23430 - http://dx.doi.org/10.3402/jev.v3.23430