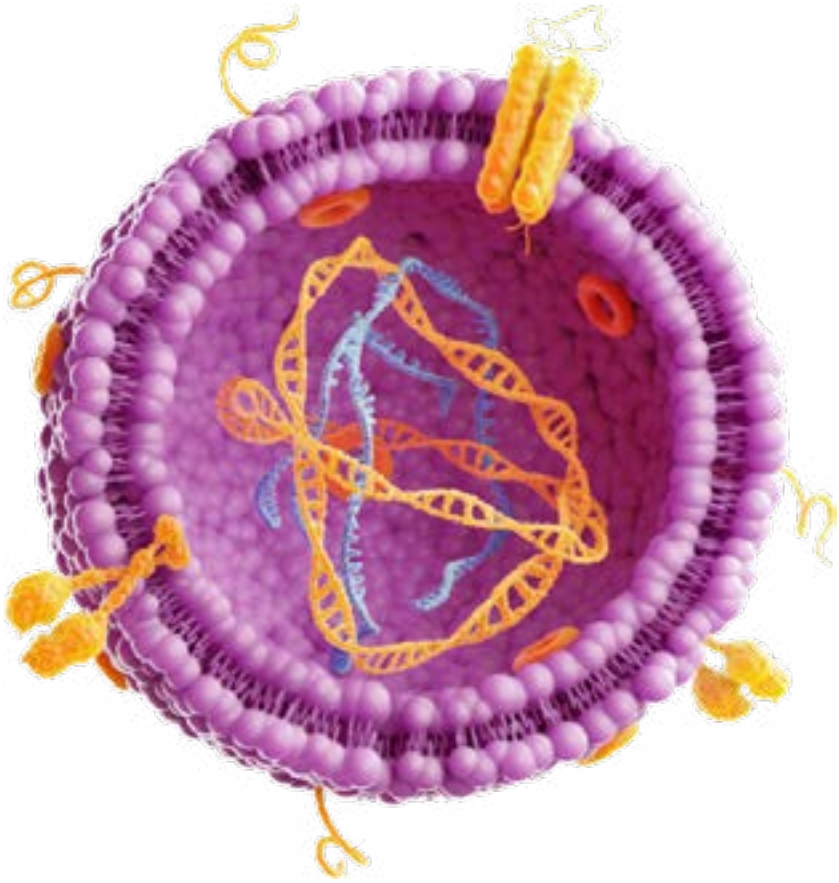
ISOLATING EVs FROM URINE USING qEV



APPLICATION NOTE

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1 / INTRODUCTION

Urine is an important biofluid containing a wide diversity of EVs, including exosomes, micro-vesicles and apoptotic bodies. Urinary EVs are a good non-invasive source of urinary proteome and transcriptome for biomarker discovery[1]. Size Exclusion Chromatography (SEC) is a widely used technique to separate complex mixtures of molecules of different sizes from various biofluids[2,3]. Izon's qEV Isolation is robust and standardised technique to purify EVs from urine samples. These can be used to reveal urological diseases or tumours as well as their progression much in advance. Izon's Automatic Fraction Collector (AFC) automates the qEV Isolation process, eliminating human error and enabling a high-precision and streamlined workflow.

2 / CONSIDERATIONS AND RECOMMENDATIONS

All qEV columns are available in one of two isolation ranges, the qEV / 35nm series and the qEV / 70nm series. For optimal recovery of particles between 35 and 350 nm a qEV / 35nm series column is recommended. For optimal recovery of particles between 70 and 1000 nm a qEV / 70nm series column is recommended.

Pre qEV sample concentration

- Urine needs to be appropriately concentrated for a detectable EV yield. The size and style of device will depend on the sample volume. Centrifuge concentrators work well for smaller samples (Amicon Ultra and Centricon Plus-70 Centrifugal filter units) and tangential or crossflow flow devices (Pellicon® (Merck Millipore) or Minimate™ (Pall Laboratory)) for larger volumes.

- Exosome purity with the 100 kDa membrane has been reported to be slightly higher as compared with 10 kDa.

- Pressure-driven tangential flow concentration is more appropriate with volumes in excess of 400 mL due to the higher flow rate achieved. Exosomal loss is only seen with the first 50-100 mL of sample.

Sample treatment

- Tamm-Horsfall Protein (THP) also known as uromodulin is the most abundant protein in normal human urine. The monomeric protein is approximately 85 kDa but in urine it can be present in large aggregates of up to several million daltons. When urine is concentrated, particularly at lower pH, THP forms a gel which has been shown to trap urinary exosomes during low speed centrifugation[4].

- THP can be removed by incubating the urine sample in dithiothreitol (DTT) 200 mg/mL at 37°C for 10 minutes followed by centrifugation at 17,000xg for 10 minutes.

qEV column loading

- Loading higher sample volumes than recommended results in a lower level of purity in the later vesicle volumes, greater overlap between protein and EV elution peaks, and higher protein levels within the EV zone. For instance, the optimal sample volume for purity on the qEVoriginal is 0.5 mL, which consistently results in vesicles eluting in the 1.5 mL EV zone.

Fig 1: Schematic representation of EV isolation from urine by qEV columns

Table 1: Izon qEV columns available and recommended volumes

Pre-concentrated urine volume	Optimal input volume	qEV column	Output volume
10-15 mL	150 µL	qEVsingle	600 µL
50-100 mL	0.5 mL	qEVoriginal	1.5 mL
200 mL	2 mL	qEV2	8 mL
500-1000 mL	10 mL	qEV10	20 mL
4-5 L	100 mL	qEV100	200 mL

3 / MATERIALS

- Urine collection tubes
- Centrifuge capable of spinning up to 17,000×g
- Micro-pipettes
- Fresh 1X PBS Solution
- Sterile 0.22 µm syringe filter
- Sterile syringe
- Izon's qEV column
- Izon's Automatic Fraction Collector (AFC)

4 / METHODS

1. Prepare fresh 1X PBS solution and filter using a sterile 0.22 µm syringe filter.

2. Equilibrate the qEV column with room-temperature PBS solution.

   a. Degassed and room temperature buffers will help to avoid air bubbles forming in the gel bed.

3. Collect urine sample and centrifuge at 180×g for 10 min at 4°C and then at 1,550×g for 20 min at 4°C. Transfer supernatant to another tube between centrifugation steps, taking care not to disrupt the pellets.

4. Concentrate the cell and debris free urine sample down to the recommended qEV load volume in Table 1.

5. Izon recommends using Amicon® Ultra coentrifugal filter units (0.5 -15) and Centricon Plus-70 centrifugal units for volumes up to 400 mL. For larger volumes, use Tangential Flow Filtration systems e.g. Pellicon® (Merck Millipore) and Minimate™ (Pall Laboratory).

6. If the concentrated sample is viscous, use the recommended DTT incubation procedure to remove Tamm-Horsfall protein prior to loading onto a qEV.

7. Affix an appropriate sized qEV for the sample volume to an AFC or qEV rack and load the sample.

   a. Be sure that the volume of the sample is appropriate for the type of qEV column used; for more information, visit www.izon.com.

8. Begin collecting the void volume and EV volume.

   a. Different samples may give slightly different elution profiles and purity, hence an initial measurement of EV concentration and protein contaminants in collected volumes is recommended.

9. After completing collection of the EV volume, flush the column with at least 1.5 column volumes of buffer before loading another sample or storing the column for future use.

10. Urinary EVs are ready for downstream applications. Izon recommends performing TRPS analysis for a standardised EV characterisation and quantification (size, concentration and charge).

5 / REFERENCES

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