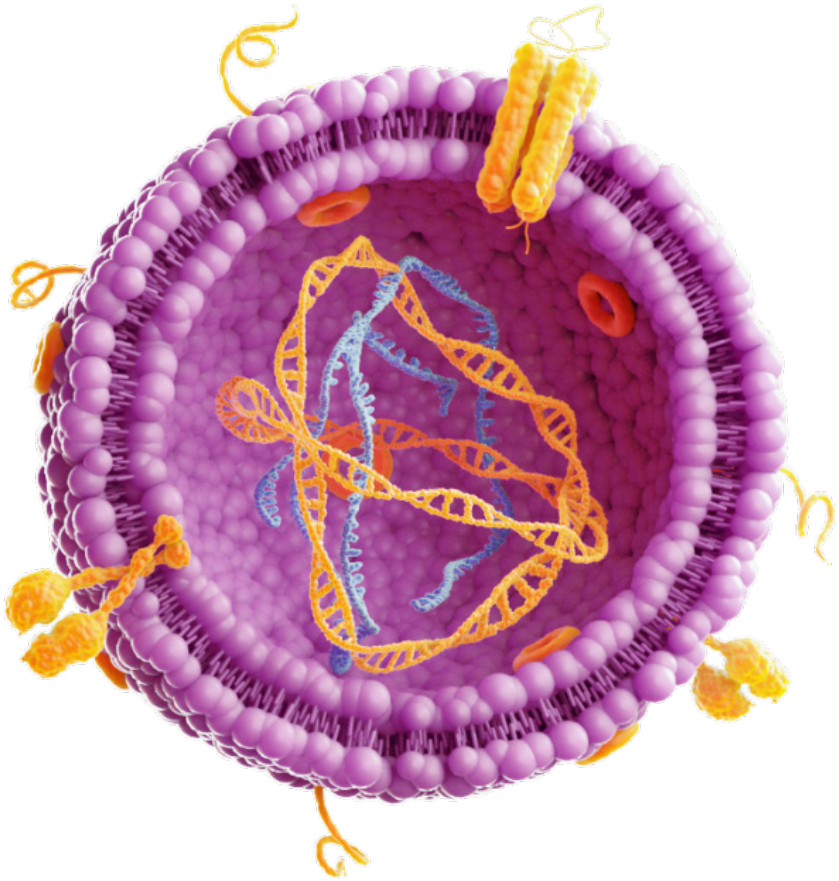
ISOLATING EVs FROM CELL CULTURE MEDIA USING qEV



APPLICATION NOTE

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1 / INTRODUCTION

Cell Cultures are imperative model systems simulating cellular processes in the laboratory. Cell Cultures can be used to diagnose diseases, screen new drugs, or produce therapeutic moieties. Extracellular Vesicles (EVs) released by cells in cell culture medium (CCM) can serve as biomarkers for the development of superior, sensitive, and minimally invasive diagnostic alternatives in modern medicine[1]. A robust and standardized method of EV isolation from conditioned media with high yield can significantly enhance their usability for research purposes. Size Exclusion Chromatography (SEC) based columns provide clean samples without affecting the structure or function of the EVs[2]. Process automation by an Automatic Fraction Collector (AFC) can further boost the reproducibility of sample isolation by reducing scope of manual errors.

2 / CONSIDERATIONS AND RECOMMENDATIONS

All qEV SMART 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.

Sample collection

EV production is cell line/cell density/incubation time dependent.

Fetal bovine serum (FBS) is the most commonly used supplement, ultracentrifugation or Tangential Field Flow (TFF) fractionation can help get rid of EVs arising from FBS.

EV isolation methods

Prior to the use of SEC, ultracentrifugation (UC) was used widely to isolate EVs. The very high forces used (up to 100,000xg), disrupted and aggregated the vesicles, invalidating much of the research. It has been observed that pressure-driven concentrating is more appropriate with volumes in excess of 400 mL due to the higher flow rate, while exosomal loss is only seen with the first 50-100 mL of CCM[3].

qEV isolation removes contaminating proteins and other small particulates from cell culture samples, leaving a pure sample of EVs equilibrated in PBS. Depending on the final CCM volume obtained, table 1 can be used to choose the right qEV for your research protocol.

Sample loading and concentration

Before loading a sample onto the column, the protein concentration in the CCM should be < 70 mg/mL.

Loading higher sample volumes results in a lower level of purity in the later vesicle volumes, greater overlap between protein and EV elution peaks, and a higher protein peak within the EV zone[4]. For instance, the optimal recommended sample volume for purity on the qEVoriginal is 500 µL, which consistently results in vesicles eluting in the 1.5 mL EV zone.

Higher volumes of CCM (> 50 ml) need to be appropriately concentrated for a greater EV yield. A centrifuge-based concentrator is the most appropriate device. Some applications may require the use of even larger volumes of CCM (4-5 L). For bioreactor broth, no pre-concentration step is required.

For most purposes including proteomics and functional assays, it is recommended to use Amicon® Ultra-4 Centrifugal Filter Unit with Ultracel-10 membrane (MWCO = 100kDa; Merck Millipore, Billerica, MA).

Fig 1: Schematic representation of EV isolation from CCM using qEV columns

Table 1: Different qEV columns available for use

CCM volume before concentration	Input qEV volume	qEV column	Output volume
< 15 mL	100-150 µL	qEVsingle	600 µL
15-100 mL	500-1000 µL	qEVoriginal	1.5 mL
100-400 mL	2 mL	qEV2	8 mL
400-1000 mL	5-10 mL	qEV10	20 mL
4-5 L	100 mL	qEV100	200 mL

3 / MATERIALS

- CCM collection apparatus
- Centrifuge capable of spinning up to 10,000×g
- Amicon® Ultra centrifugal filters
- TFF fractionation system (if required)
- 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. Affix the AFC as per the user manual and load the sample on an appropriate qEV column described in table 1.

   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

A. Isolation of EVs from CCM sample using Izon's qEVsingle SEC column

1. Carefully remove the cell culture supernatant (<15 mL).
2. Centrifuge the supernatant at 500×g for 10 min and then 10,000×g for 10 min.
3. Spin the supernatant using Amicon® Ultra-4 or Amicon® Ultra-15 Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore, Billerica, MA).
4. Concentrate to a final volume of 100-150 µL by repeated centrifugation.

Note: If required, adjust the final volume to 150 µL with 1X PBS.

5. Overlay the adjusted input volume on the qEVsingle column.
6. Immediately start collecting the void volume (1 mL).
7. Collect the EV volume (600 µL) and concentrate using Amicon® Ultra-4 or Amicon® Ultra-15 Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore, Billerica, MA).
8. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for EV characterization and quantification.

B. Isolation of EVs from CCM sample using Izon's qEVoriginal SEC column

1. Obtain the supernatant (15-100 mL) after removing cell debris.
2. Follow steps 2 and 3 described in section A.
3. Concentrate the clarified CCM to a final volume of 500-1000 µL by repeated centrifugation.
4. Place 500-1000 µL of concentrated sample on a qEVoriginal and let it elute with PBS.
5. Immediately start collecting the void volume (3 mL).
6. Collect the EV volume (1.5 mL) and concentrate using Amicon® Ultra-4 or Amicon® Ultra-15 Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore, Billerica, MA).
7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for EV characterization and quantification.

C. Isolation of EVs from CCM sample using Izon's qEV2 SEC column

1. Obtain the supernatant (100-400 mL) after removing cell debris.
2. Follow steps 2 and 3 described in section A.
3. Concentrate the clarified CCM to a final volume of 2ml by repeated centrifugation.
4. Place 2ml of concentrated sample on a qEV2 and let it elute with PBS.
5. Immediately start collecting the void volume (14.25 mL).
6. Collect the EV volume (8 mL) and concentrate using Amicon® Ultra-4 or Amicon® Ultra-15 Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore, Billerica, MA).
7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for EV characterization and quantification.

D. Isolation of EVs from CCM using Izon's qEV10 SEC column

1. Obtain the supernatant (400-1000 mL) after removing cell debris.
2. Centrifuge the supernatant at 500×g for 10 min and then 10,000×g for 10 min.
3. Concentrate the clarified CCM to a final volume of 5-10 mL by techniques such as Jumbosep™ Centrifugal Devices (Pall Corporation, New York) or Tangential Flow Filtration (TFF) (Minimate™ Tangential Flow Filtration System, Pall Corporation, New York) working at a flow rate of 30-80 mL/min. TFF can sample volumes of up to 1 L or more and efficiently concentrate to as little as 5 mL.
4. Place the concentrated sample on a qEV10 and let it elute with PBS.
5. Immediately start collecting the void volume (20 mL).
6. Collect the EV volume (20 mL) and concentrate using Amicon® Ultra-4 or Amicon® Ultra-15 Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore, Billerica, MA).
7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for EV characterization and quantification.

E. Isolation of EVs from CCM using Izon's qEV100 SEC column

1. Obtain the supernatant (4-5 L) after removing cell debris.
2. Centrifuge the supernatant at 500×g for 10 min and then 10,000×g for 10 min.
3. Concentrate clarified CCM to a final volume of 100 mL by techniques such as Jumbosep™ Centrifugal Devices (Pall Corporation, New York) or Tangential Flow Filtration (TFF) (Minimate™ Tangential Flow Filtration System, Pall Corporation, New York) working at a flow rate of 30-80 mL/min. TFF can sample volumes of up to 1 L or more and efficiently concentrate to as little as 5 mL.
4. Place the concentrated sample on a qEV100 and let it elute with PBS.
5. Immediately start collecting the void volume (200 mL).
6. Collect the EV volume (200 mL) and concentrate using Amicon® Ultra-4 or Amicon® Ultra-15 Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore, Billerica, MA).
7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for EV characterization and quantification.

5 / REFERENCES

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