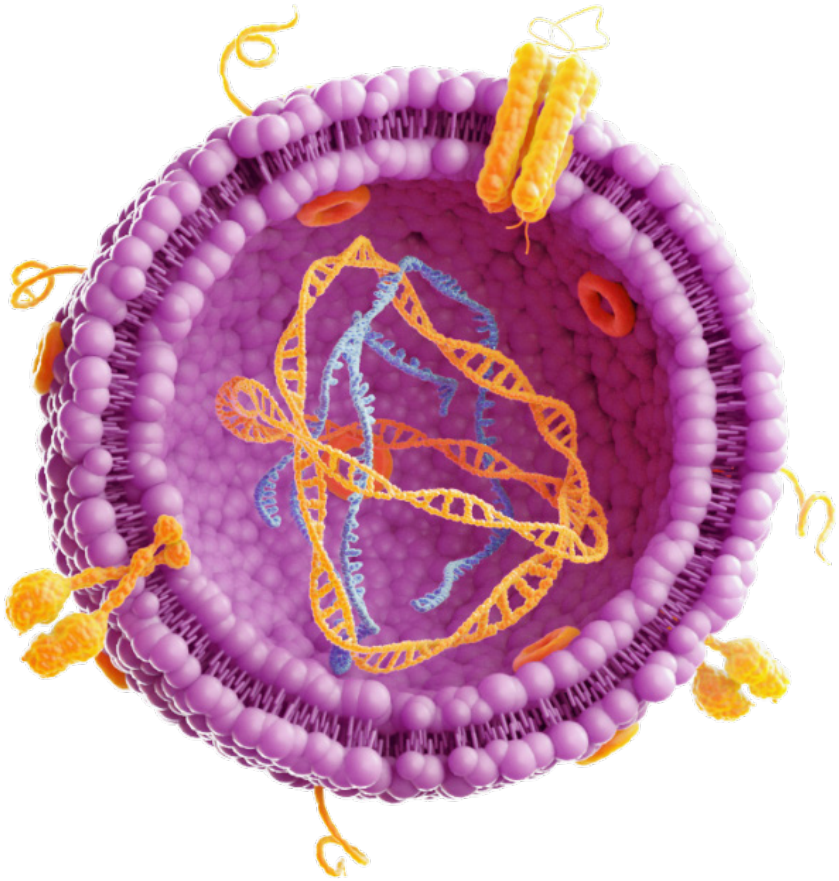
ISOLATING EVs FROM CELL CULTURE MEDIA USING qEV



APPLICATION NOTE

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1 / INTRODUCTION

Cell cultures are crucial models used to simulate biological processes in the laboratory and explore different research areas, including the study of extracellular vesicles (EVs) and relevant applications. The isolation of EVs from cells in cell culture conditioned medium (CCM) has many advantages; cell culture systems can be controlled and optimised, and present opportunities for large-scale production of CCM EVs.

Extracellular vesicles (EV) are the broad classification of membrane-derived entities produced by a variety of activated and apoptotic cell types. The term "EV" includes exosomes, microvesicles (sometimes called microparticles), oncosomes and other vesicles that are defined by their cellular origin, size, and surface markers[1]. They are used to transmit signals between cells[1], and their ability to transport molecules to specific target cell populations make them attractive for diagnostic and therapeutic development.

Cell cultures are crucial model systems used to simulate cellular and biological processes in research laboratories. Cell cultures systems have been used as platforms for research in many different areas, like understanding biological processes, investigating diagnostic potential, drug development, or the production of biologicals for nanomedicine. The isolation of EVs released by cells in CCM can present many advantages: production of CCM EVs is a scalable process, where specific conditions can be adjusted in smaller or larger scales; mass production of EVs is enabled when the procedure is scaled up or automated, generating litres of EV-containing CCM[2]; several variables in cell culture systems can be controlled, studied and optimised to produce desired outcomes.

A robust and standardised high-yield method of EV isolation from CCM can significantly enhance the usability of EVs for applied or research purposes. By harnessing size exclusion chromatography (SEC), qEV columns provide clean EV samples without affecting EV structure or function.[3-5] The reproducibility of qEV-based sample isolation can be boosted using the Automatic Fraction Collector (AFC), which introduces an element of automation to further reduce the scope of manual errors.

2 / CONSIDERATIONS AND RECOMMENDATIONS

Although a CCM sample may seem less complex than a blood sample for EV isolation, it does have its own challenges: 1) to successfully culture cells and obtain CCM without any microbial contamination and in a safe manner to the user, specialised facilities and equipment are required; 2) microbial contamination of CCM not only affects the cells but also the resulting EV preparation, as microbes also release EVs; 3) the most commonly used supplement for cell culture is Fetal Bovine Serum (FBS) which contains significant amounts of cow-derived EVs that contaminate CCM EVs.

A summary flowchart showing general steps in CCM progression and EV isolation is shown in Figure 1.

Sample collection

The EV composition and yield present in CCM depends on many variables: cell line, cell density, media composition, incubation conditions (time, temperature, oxygen levels, etc).

A way to avoid FBS EVs contaminating CCM EVs, is to remove FBS-EVs from FBS used for culture with methods such as ultracentrifugation, microfiltration or ultrafiltration.

CCM concentration

As the EV concentration in CCM samples is not high, it is recommended to concentrate CCM samples to improve recovery of the following qEV isolation. Concentration of CCM sample not only enriches EVs (increased EV counts per unit of volume) but also reduces CCM volume to a volume suitable for qEV isolation columns and removes a part of contaminating proteins.

In this way, large volumes of CCM (> 50 mL) require concentration for a greater EV yield. Ultrafiltration devices like centrifugal concentrators are mostly used to concentrate volumes < 50 mL, however for larger CCM volumes (> 200 mL) devices using cross flow filtration (CFF, also known as tangential flow filtration, TFF).

Ultrafiltration devices commonly used for concentration of EV in CCM samples are based on pore sizes with a molecular weight cut-off (MWCO) of around 100, 300 or 750 kDa.

qEV isolation

There is a range of qEV isolation columns suited to different EV size ranges and sample volumes to match various research needs for CCM EVs.

The new Gen 2 qEV columns are made with a proprietary agarose resin, which delivers a more purified extracellular vesicle (EV)-containing eluate, removing more contaminating protein than Legacy (containing previous resin) qEV columns.

Both Legacy and Gen 2 are available now in the existing 35 nm and 70 nm series, which have optimum recovery ranges of 35-350 nm and 70-1000 nm, respectively. More information on qEV columns used for CCM EVs can be found in Table 1.

It is important to follow the recommendations for sample input volumes for each qEV column (Table 1). Loading larger sample volumes results in a lower level of purity in later Purified Collection Volumes (PCV), greater overlap between protein and EV elution peaks, and a higher protein peak within the EV zone[4].

Also, if working with highly concentrated CCM samples, it is recommended to check the protein concentration, as the protein concentration of CCM input sample in the qEV column should be < 70 mg/mL.

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

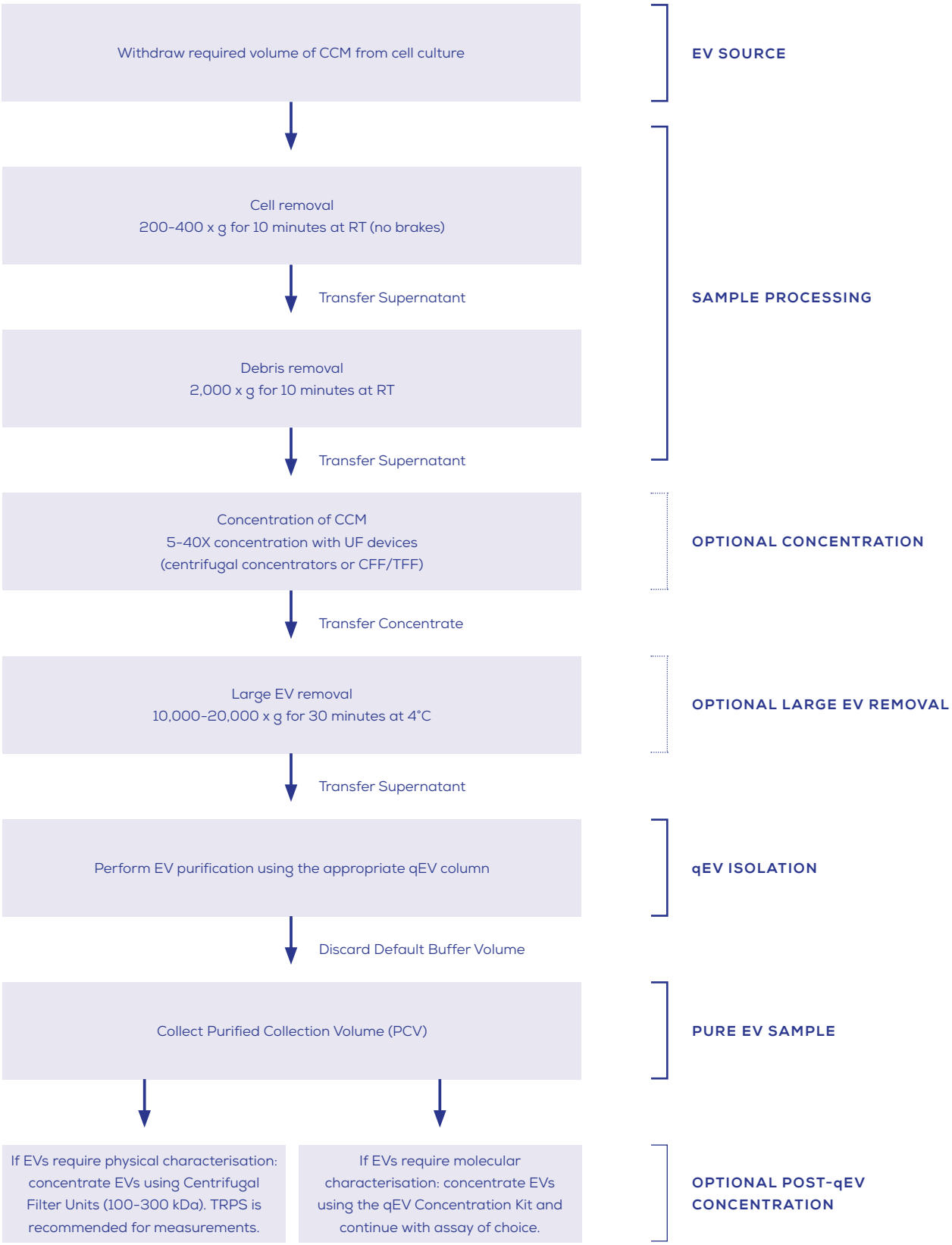

Table 1. qEV columns available for use with CCM samples

SUGGESTED CCM VOLUME BEFORE CONCENTRATION	INPUT qEV VOLUME	qEV COLUMN	PURIFIED COLLECTION VOLUME (PCV)
< 15 mL	150 µL	qEVsingle [a]	Legacy – 600 µL
15 – 100 mL	500 µL	qEVoriginal [b]	Legacy – 1.5 mL Gen 2 – 1.6 mL
50 – 200 mL	1 mL	qEV1 [c]	Gen 2 – 2.8 mL
100 – 300 mL	2 mL	qEV2 [a]	Legacy – 8 mL
300 – 1000 mL	10 mL	qEV10 [a]	Legacy – 20 mL
4 – 5 L	100 mL	qEV100 [a]	Legacy – 200 mL

a / Legacy only, b / Gen 2 & Legacy, c / Gen 2 only

3 / MATERIALS

- CCM collection apparatus
- Centrifuge capable of spinning up to 10,000×g
- Ultrafiltration devices (Amicon® Ultra centrifugal filters or CFF/TFF device)
- Micro-pipettes
- Fresh 1X PBS Solution
- Sterile 0.22 µm syringe filter
- Sterile syringe
- Izon's qEV column
- Izon's Automated Fraction Collector (AFC)

4 / METHODS

General considerations for qEV isolation

1. Prepare fresh 1X PBS solution and filter using a sterile 0.22 µm syringe filter.
2. Affix the AFC as per the user manual, with an appropriate qEV column. For more information visit www.izon.com
3. Equilibrate the qEV column with room-temperature PBS solution following instructions in each qEV column user manual.

    a. Be sure that the volume of the sample is appropriate for the type of qEV column used (see Table 1).

A. Isolation of EVs from CCM using the qEVsingle column

1. Carefully remove the cell culture supernatant (<15 mL).
2. Centrifuge the supernatant (SN) at 200 ×g for 10 min and then transfer SN onto new tube and centrifuge 2,000×g for 10 min.
3. Concentrate the clarified CCM by using Amicon® Filter Units (MWCO = 100kDa; Merck Millipore), until a final volume of 150 µL is reached.
4. Overlay the concentrated CCM input volume on the qEVsingle column.
5. Immediately start collecting the Buffer Volume (1 mL) and discard.
6. Collect EVs eluted in PCV volume (600 µL) and concentrate if needed using Amicon® Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore).
7. EVs are ready for downstream applications. Izon recommends performing TRPS analysis for the physical characterisation and quantification of EVs.

B. Isolation of EVs from CCM using the qEVoriginal (Legacy) column

1. Carefully remove the cell culture supernatant (15 to 100 mL).
2. Centrifuge the SN at 200 ×g for 10 min and then transfer SN onto new tube and centrifuge 2,000×g for 10 min.
3. Concentrate the clarified CCM by using Amicon® Filter Units (MWCO = 100kDa; Merck Millipore) or a CFF/TFF device, until a final volume of 500 µL is reached.
4. Overlay the concentrated CCM input volume on the qEVoriginal column.
5. Immediately start collecting the Buffer Volume (2.7 mL) and discard.

6. Collect EVs eluted in PCV volume (1.5 mL) and concentrate if needed using Amicon® Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore).

7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for the physical characterisation and quantification of EVs.

C. Isolation of EVs from CCM using the qEV1 column

1. Carefully remove the cell culture supernatant (50 to 200 mL).

2. Centrifuge the supernatant (SN) at 200 ×g for 10 min and then transfer SN onto new tube and centrifuge 2,000×g for 10 min.

3. Concentrate the clarified CCM by using Amicon® Filter Units (MWCO = 100kDa; Merck Millipore), until a final volume of 1 mL is reached.

4. Overlay the concentrated CCM input volume on the qEV1 column.

5. Immediately start collecting the Buffer Volume (4 mL) and discard.

6. Collect EVs eluted in PCV volume (2.8 mL) and concentrate if needed using Amicon® Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore).

7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for the physical characterisation and quantification of EVs.

D. Isolation of EVs from CCM using the qEV2 column

1. Carefully remove the cell culture supernatant (100-300 mL).

2. Centrifuge the SN at 200 ×g for 10 min and then transfer SN onto new tube and centrifuge 2,000×g for 10 min.

3. Concentrate the clarified CCM by using Amicon® Filter Units (MWCO = 100kDa; Merck Millipore) or a CFF/TFF device, until a final volume of 2 mL is reached.

4. Overlay the concentrated 2 mL CCM input volume on the qEV2 column.

5. Immediately start collecting the Buffer Volume (14.1 mL) and discard.

6. Collect EVs eluted in PCV volume (6 mL) and concentrate if needed using Amicon® Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore).

7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for the physical characterisation and quantification of EVs.

E. Isolation of EVs from CCM using the qEV10 column

1. Carefully remove the cell culture supernatant (300-1000 mL).

2. Centrifuge the SN at 200 ×g for 10 min and then transfer SN onto new tube and centrifuge 2,000×g for 10 min.

3. Concentrate the clarified CCM by using special large Centrifugal devices or a CFF/TFF device (can process litres of samples efficiently), until a final volume of 10 mL is reached.

4. Overlay the concentrated 10 mL CCM input volume on the qEV10 column.

5. Immediately start collecting the Buffer Volume (20 mL) and discard.

6. Collect EVs eluted in PCV volume (15 mL) and concentrate if needed using Amicon® Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore).

7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for the physical characterisation and quantification of EVs.

F. Isolation of EVs from CCM using the qEV100 column

1. Carefully remove the cell culture supernatant (4-5 L).

2. Centrifuge the SN at 200 ×g for 10 min and then transfer SN onto new tube and centrifuge 2,000×g for 10 min.

3. Concentrate the clarified CCM by using CFF/TFF devices (can process litres of samples efficiently), until a final volume of 100 mL is reached.

4. Overlay the concentrated 100 mL CCM input volume on the qEV100 column.

5. Immediately start collecting the Buffer Volume (150 mL) and discard.

6. Collect EVs eluted in PCV volume (200 mL) and concentrate if needed using Amicon® Centrifugal Filter Units (MWCO = 100kDa; Merck Millipore).

7. The EVs are now ready for downstream applications. Izon recommends performing TRPS analysis for the physical characterisation and quantification of EVs.

5 / REFERENCES

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