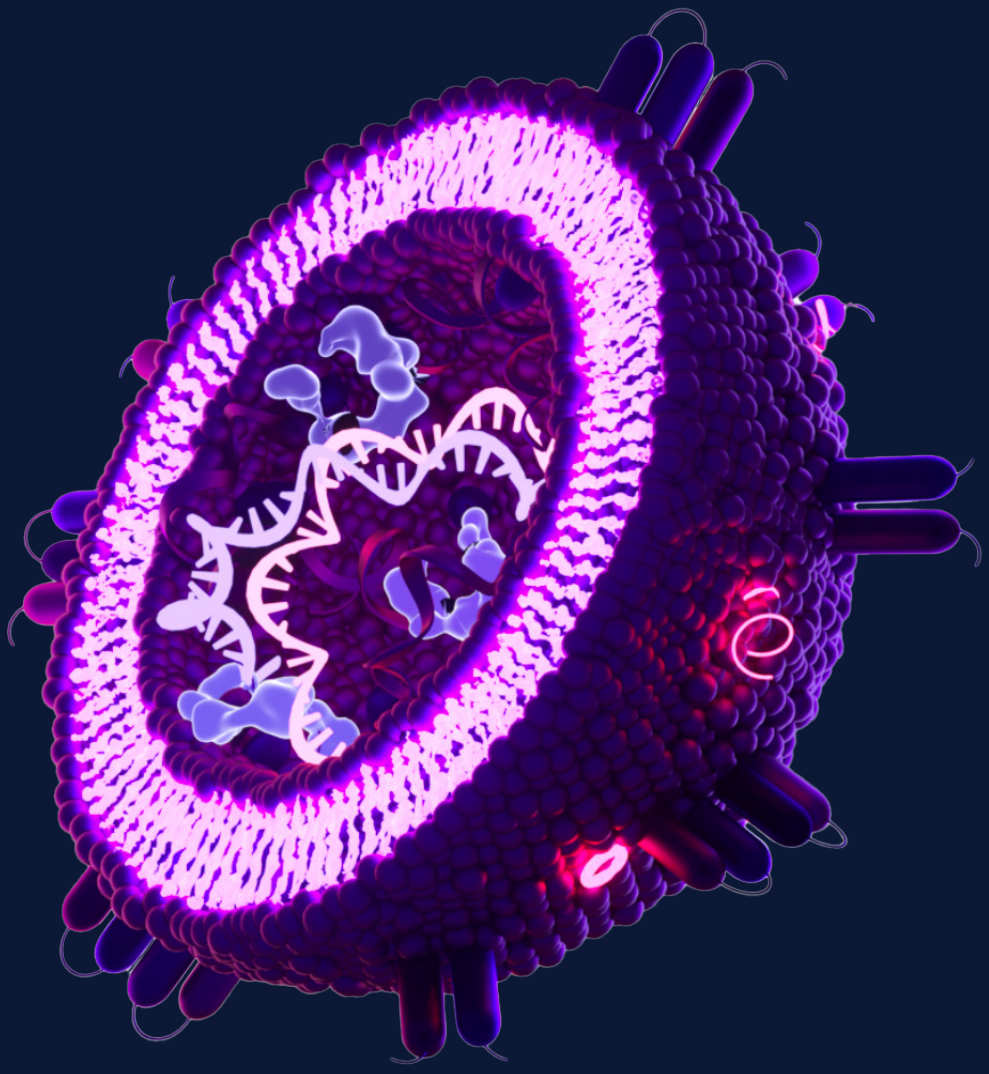
ISOLATING EVs FROM CELL CULTURE MEDIA USING qEV COLUMNS



APPLICATION NOTE

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1 / INTRODUCTION

Cell cultures are crucial *in vitro* research models which enable the production of extracellular vesicles (EVs) for fundamental research, as well as the exploration of EV-based therapeutics, cosmetics, and diagnostics. The isolation of EVs from conditioned media (CM) has many advantages as a study model, allowing fine control over cell culture conditions and facilitating the large-scale production of EVs.

'Extracellular vesicles' is a blanket term for membrane bilayer vesicles produced and secreted by cells. EV types are differentiated from one another by their biogenesis, and include exomeres, exosomes, microvesicles (also called microparticles), oncosomes and many others (Figure 1). Different types of EVs can overlap within any given size range.

One of the main functions of EVs is to transmit signals to targets cells[1], making them of interest within the context of therapeutics. As their cargo is impacted by intrinsic (e.g., genetics) and extrinsic (e.g., disease state) factors, EVs are also an important player in diagnostics.

However, the translation of EVs to clinical applications is only possible with a robust and standardised method for the isolation of high-yield, highly pure EVs from CM. By harnessing size exclusion chromatography (SEC), qEV columns provide clean EV samples in a reproducible manner. Importantly, SEC is a gentle method, which means EVs are isolated without using strong forces which could compromise EV structure or function.[2-4] The reproducibility of qEV isolates can be boosted by the Automatic Fraction Collector (AFC) which introduces automation to improve throughput and minimise manual errors.

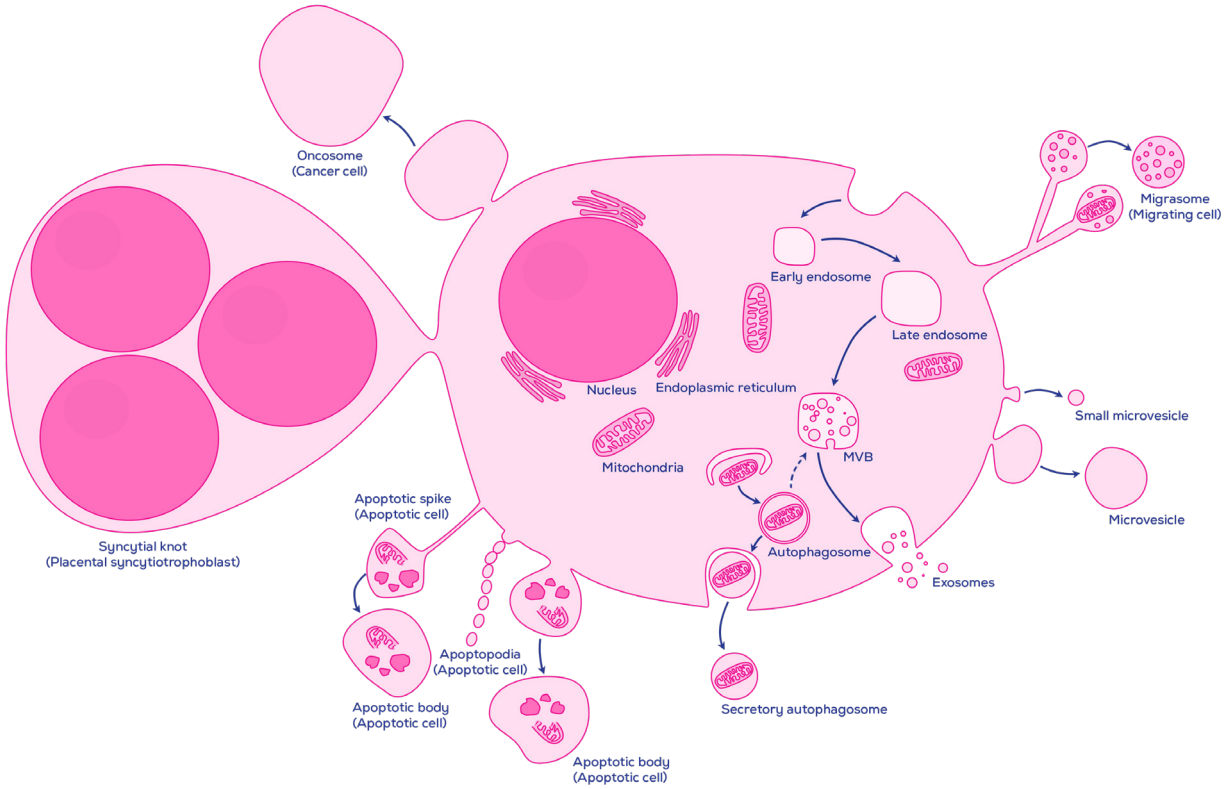

Figure 1. A non-exhaustive representation of many different types of extracellular vesicles (EVs).

2 / CONSIDERATIONS AND RECOMMENDATIONS

Although a CM sample may seem less complex than a blood sample, it poses its own challenges for isolating EVs:

1. Microbial contamination
2. Fetal Bovine Serum (FBS)-derived EV contamination
3. Large volumes
4. Dilute EVs

However, there are ways to tackle these challenges.

Sample generation and collection

The composition and yield of CM EVs depends on many variables, including cell line, cell density, media composition, treatments, and incubation conditions. These conditions must be optimised for your specific circumstances.

To tackle microbial contamination, cells should always be cultured under sterile conditions. Whilst antibiotics can be added to cell culture, they do alter cell behaviour and EV composition[5], and may contaminate EV isolates. As such, good sterile technique is essential and antibiotic-free culture is preferred. For an industrial scale, sterility can be aided by pairing automation and clean rooms.

EVs can be removed from FBS using ultracentrifugation, microfiltration or ultrafiltration, or EV-depleted FBS can be purchased. You could also choose to omit FBS.

Concentration of conditioned media prior to EV isolation

As EVs in CM are relatively dilute, concentration is highly recommended. If samples are not concentrated, then the relatively low concentration of EVs within the sample will subsequently result in a very dilute EV isolate. Concentration not only enriches EVs (by increasing EV counts per unit of volume), but it also reduces the CM volume, making it more suitable for isolation with qEV columns. Of note, our 'off the shelf' qEV Gen 2 columns can accommodate a range of volumes, with the largest column suitable for 100 mL of sample loading volume.

Some researchers, however, may choose not to concentrate their EVs prior to qEV isolation. In that case, and for therapeutics customers seeking to scale their isolation, qEV PurePath for Therapeutics may be of interest; where we collaborate with you to create much larger columns for isolating at scale.

For volumes under 50 mL, we recommend dead-end filtration (e.g., Amicon® Ultra centrifugal filters), whilst tangential flow filtration (TFF) is more suited to concentrating larger samples. EVs can be concentrated using pore sizes with a molecular weight cut-off (MWCO) of 100, 300 or 750 kDa. These pore sizes permit the passage of some proteins into the filtrate, which prevents protein from becoming too concentrated and therefore helps ensure samples are appropriate for subsequent qEV isolation.

For isolating large volumes of CM – even for starting volumes as high as 2000 L – get in touch with our qEV PurePath team to discuss bespoke workflow design and large customised columns.

Optional processing steps to remove or enrich EV subpopulations prior to qEV isolation

If isolating a specific size range of EVs is of interest, an optional centrifugation step can be introduced prior to qEV isolation to remove small or large EVs. It is important to note that the categories of large and small EVs reflect sedimentation rates that occur at different centrifugation speeds, rather than specific subpopulations of EVs. Furthermore, additional centrifugation steps introduce a level of variability that will be unacceptable to some, and a level of manual labour that will not be practical in many settings. Therefore, these steps are not included as core recommendations in the main protocol. However, the following steps can be followed to approximately separate large and small EVs prior to qEV isolation, should this be of interest:

- Large EVs: Centrifuge the EV-containing sample at 10,000-20,000 x g for approximately 30 minutes, then suspend the pellet (containing large EVs) to an appropriate buffer volume for qEV isolation.
- Small EVs: After following the step above, the supernatant can be loaded to a qEV column, to separate small EVs from other constituents.

qEV isolation

To match the various needs of researchers working with CM EVs, Gen 2 qEV isolation columns are available to suit different EV size ranges and sample volumes.

Gen 2 qEV columns are made with a customised, high-performance agarose resin which delivers a highly purified EV isolate, removing more contaminating protein (~99%) than our previous Legacy qEV columns. Currently, both Legacy* and Gen 2 columns are available in the 35 nm and 70 nm Series, which have optimum recovery ranges of 35-350 nm and 70-1000 nm, respectively.

Suitable CM input volumes for qEV columns are presented in Table 1. Loading sample volumes outside of the recommended ranges may compromise results; e.g., exceeding recommended sample volumes for the column results in a lower level of purity, greater overlap between protein and EV elution peaks, and a higher protein peak within the EV zone.[4]

If working with highly concentrated CM samples, it is possible to reach very high protein levels which may impact upon the performance of the qEV columns. For reference, qEV columns are routinely used with plasma samples, which have protein levels in the range of 40 - 80 mg/mL.[6,7] If you are losing large quantities of EVs, or co-isolate proteins in your qEV EV isolate, then we recommend that you either dilute your sample, reduce the protein concentration, or alter your concentration procedure to prevent unnecessary protein enrichment (e.g., using larger pore sizes during filtration).

*Legacy qEV columns are being phased out, with production to end in 2023.

3 / MATERIALS

- CM collection apparatus (e.g., pipettes for smaller scale studies or an automated system for bioreactors)
- Centrifuge capable of spinning up to 500 x g or, for optional steps, up to 10,000 × g (ideally temperature controlled)
- Ultrafiltration devices (e.g., Amicon® Ultra centrifugal filters or CFF/TFF device)
- Fresh 0.22 µm filtered 1x PBS
- qEV column
- Optional: Automatic Fraction Collector (AFC)

Table 1. qEV columns available for use with CM samples

SUGGESTED CONDITIONED MEDIA VOLUME BEFORE CONCENTRATION (SAMPLE DEPENDENT)	INPUT qEV VOLUME*	qEV COLUMN	PURIFIED COLLECTION VOLUME (PCV)	
			LEGACY	GEN 2**
< 15 mL	150 µL	qEVsingle	600 µL	680 µL
15 - 100 mL	500 µL	qEVoriginal	1.5 mL	1.6 mL
50 - 200 mL	1 mL	qEV1	N/A	2.8 mL
100 - 300 mL	2 mL	qEV2	6 mL	8 mL
300 - 1000 mL	10 mL	qEV10	15 mL	20 mL
4 -5 L	100 mL	qEV100	200 mL	200 mL

*qEV Columns are optimised to maximise purity using human plasma samples.

**Default PCV specified for use of Gen 2 columns on the Automatic Fraction Collector. The default recommended PCV provides a balance of EV recovery and high purity. Consult your qEV user manual for more information on recommended parameters.

4 / METHODS

- Ensure that the volume of your sample is appropriate for the type of qEV column used (Table 1).
- Follow the procedure below, making sure that you follow the relevant qEV user manual for all qEV steps.

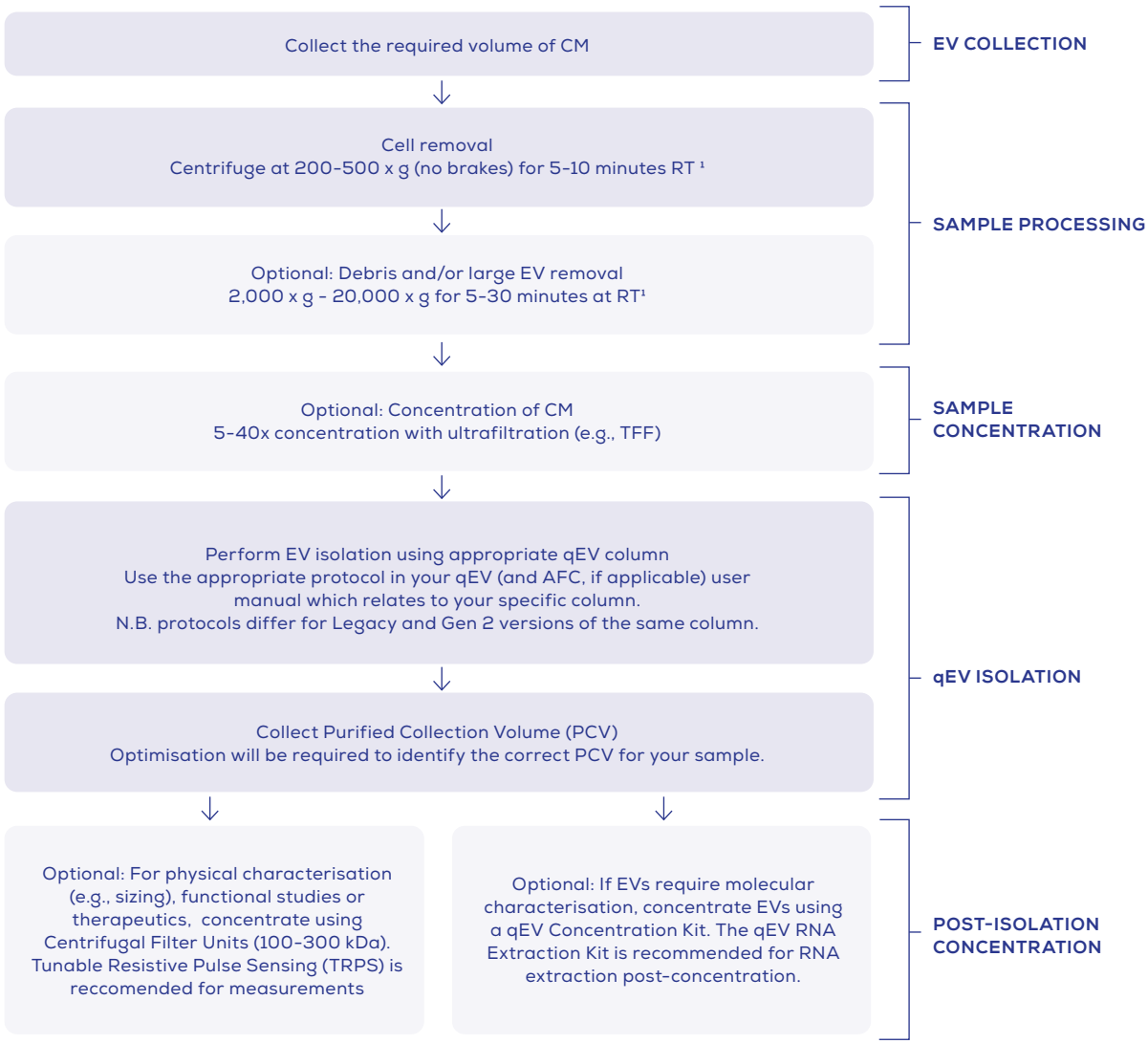

Figure 2. A schematic method of isolating extracellular vesicles (EVs) from conditioned media (CM).

5 / REFERENCES

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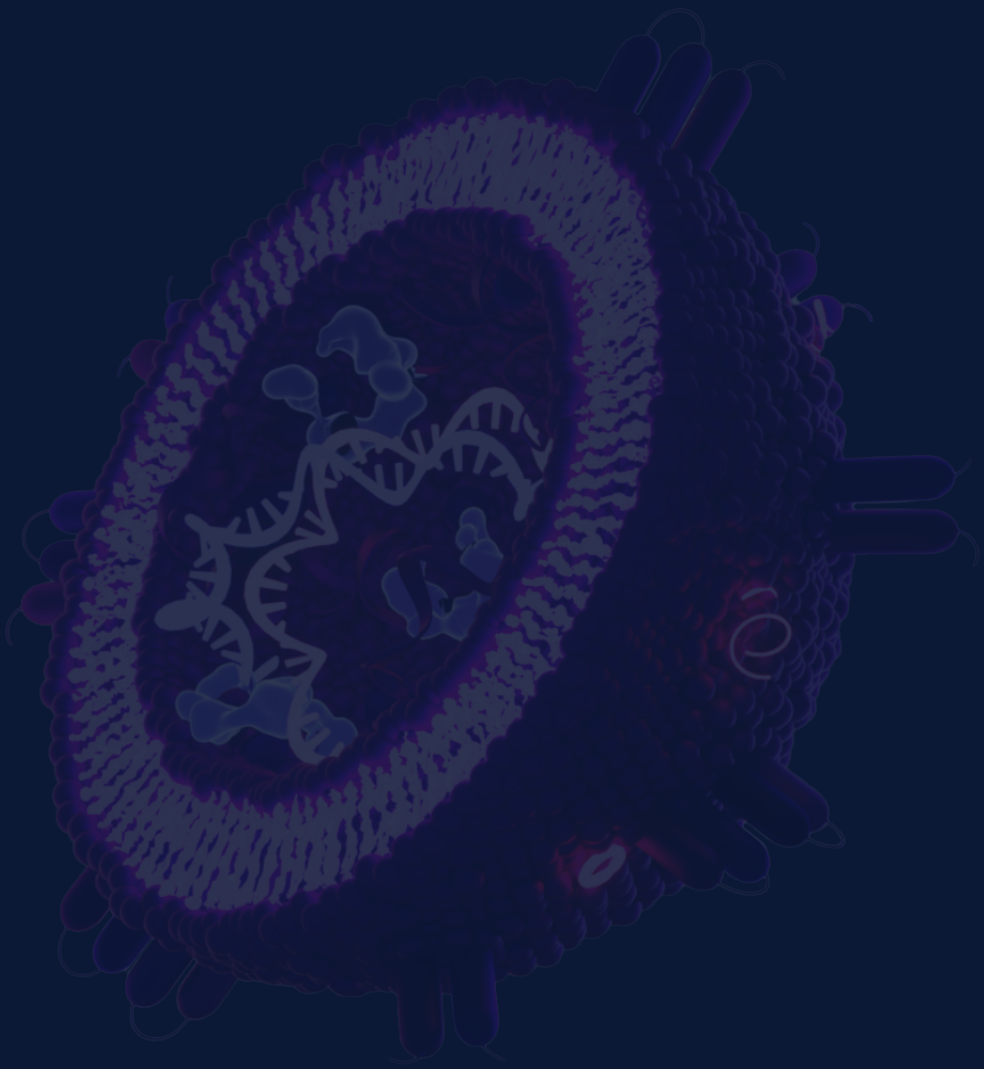

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