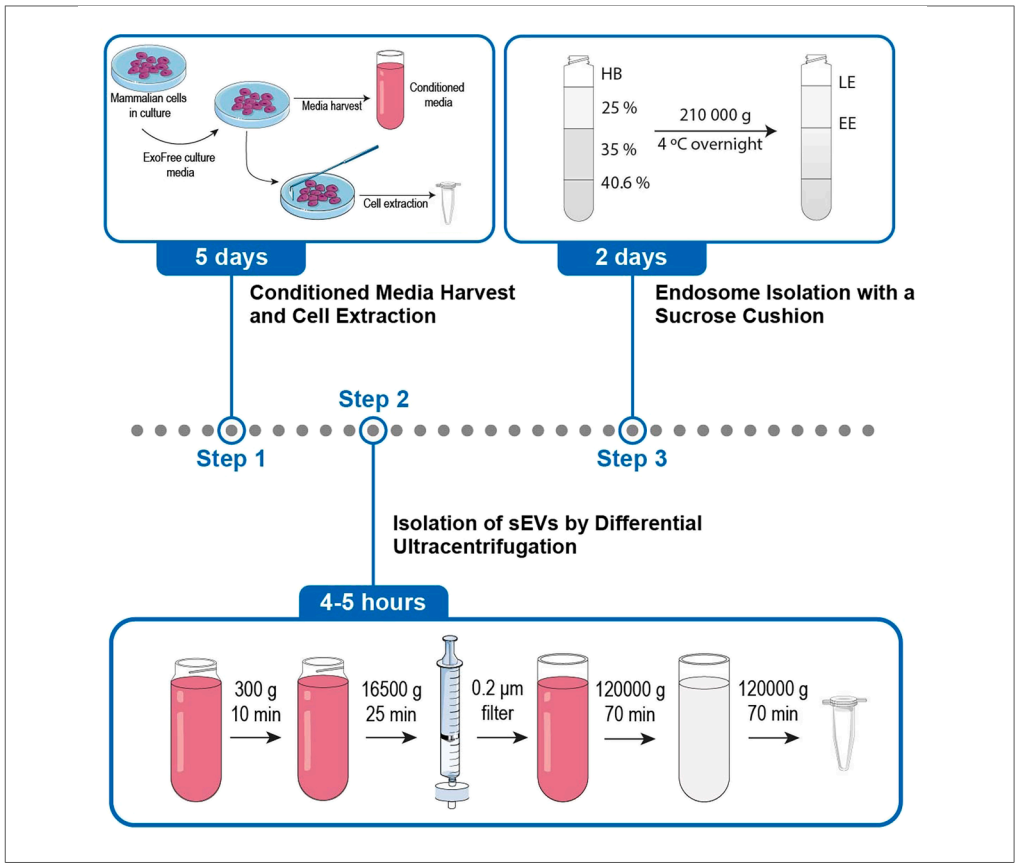
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol to isolate endosomal and small extracellular vesicles from cultured cells through ultracentrifugation



Here, we present a protocol to isolate endosomal fractions using sucrose-density gradient ultracentrifugation and to recover small extracellular vesicles (sEVs), enriched in exosomes, using sequential ultracentrifugation from mammalian cell lines. This combined approach enables the separation and analysis of early endosome (EE), late endosome (LE), and sEV fractions. We provide detailed procedures for cell culture preparation, conditioned media collection, differential centrifugation, gradient layering, and fraction purification, facilitating downstream characterization and functional assays.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Maria Nolasco Domingues, Luís Palhinhas, Paulo Pereira, João Vasco Ferreira

paulo.pereira@nms.unl.pt (P.P.)
joao.ferreira@nms.unl.pt (J.V.F.)

Highlights

Isolation of small extracellular vesicles with a sucrose cushion

Isolation of early and late endosomes with a sucrose gradient

Purification of early and late endosome fractions

Domingues et al., STAR Protocols 7, 104531
June 19, 2026
https://doi.org/10.1016/j.xpro.2026.104531

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Protocol to isolate endosomal and small extracellular vesicles from cultured cells through ultracentrifugation

Maria Nolasco Domingues,[1,2] Luís Palhinhas,[1,2] Paulo Pereira,[1,*] and João Vasco Ferreira[1,3,4,*]

[1]iNOVA4Health, NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade NOVA de Lisboa, Lisbon, Portugal

[2]These authors contributed equally

[3]Technical contact

[4]Lead contact

*Correspondence: paulo.pereira@nms.unl.pt (P.P.), joao.ferreira@nms.unl.pt (J.V.F.)
https://doi.org/10.1016/j.xpro.2026.104531

SUMMARY

Here, we present a protocol to isolate endosomal fractions using sucrose-density gradient ultracentrifugation and to recover small extracellular vesicles (sEVs), enriched in exosomes, using sequential ultracentrifugation from mammalian cell lines. This combined approach enables the separation and analysis of early endosome (EE), late endosome (LE), and sEV fractions. We provide detailed procedures for cell culture preparation, conditioned media collection, differential centrifugation, gradient layering, and fraction purification, facilitating downstream characterization and functional assays.

For complete details on the use and execution of this protocol, please refer to Ferreira et al.[1]

BEFORE YOU BEGIN

This protocol describes the isolation of enriched fractions of EE, LE and exosome enriched sEVs, from mammalian cells in culture. The fractions can be used for downstream assays including Western Blotting, Proteomics, Electron Microscopy and additional *in vitro* assays.

The day before completing the protocol, prepare all required solutions and store them at 4°C, including homogenization buffer, sucrose stock solutions, imidazole buffer and PBS. Please also account for the pre-chilling time of the centrifuges to ensure they are at 4°C when required.

Innovation

This protocol introduces optimization steps to the isolation of endosomes and sEVs, by refining multiple stages that directly impact purity and reproducibility. We have optimized cell homogenization[2] and made systematic adjustment of centrifugation times and speeds, sample volumes, and the inclusion of additional washing steps.[3] The workflow further integrates principles from density gradient–based separation of endocytic compartments,[4,5] and sucrose cushion for exosome purification.[5,6] By consolidating these optimized steps into a single, coherent workflow, the protocol enables the parallel isolation of sEVs and their cytoplasmic counterparts' populations from the same cell culture, enhancing comparability while improving overall yield, purity, and reproducibility.

ExoFree fetal bovine serum

Timing: Overnight (15–16 h)

This serum is crucial for any method of isolating EVs from culture media, since removing the bovine-derived EVs naturally present in FBS ensures that the isolated EVs fraction is only produced by the cells in culture.

1. Centrifuge FBS at 120000 *g*, overnight (15–16 h) at 4°C in a SW 32 Ti rotor;
2. Collect the supernatant to a sterile tube;

⚠ CRITICAL: When decanting the tube, pour in one single, continuous slow movement, leaving the last milliliters immediately above the pellet, to avoid disturbing it.

3. Filter the supernatant with a 0.2 μm vacuum filter, inside the sterile flow chamber, and collect the filtrate to a new sterile tube;

Cell culture

Timing: 1–2 days after full confluency

4. Add around $5x10^6$ and/or appropriate number of cells to 150 mm x 25 mm polystyrene culture dish, with 20 mL of culture media. Let cells reach full confluency;
5. Remove the cell culture media and wash the cells with 10 mL of PBS;
6. Add 20 mL ExoFree culture media for 24–48 h.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
DMEM with Glutamine	Biowest	L0102
Fetal Bovine Serum	Biowest	S1810
Penicillin-Streptomycin	Biowest	L0022
Non-Essential Aminoacids	Biowest	X0557
Sucrose	VWR	27483.294
Imidazole	Sigma-Aldrich	8.14223
EDTA	VWR	20294.294
Iodoacetamide	ThermoFisher	122270250
Cycloheximide	Sigma-Aldrich	C1988
Phenylmethylsulfonyl fluoride	ThermoFisher	36978
Protease Cocktail Inhibitor	Sigma-Aldrich	P8340
KCl	VWR	26764.298
Na_2HPO_4	VWR	28026.361
KH_2PO_4	VWR	26936.320
NaCl	VWR	27800.460
Deuterium Oxide (D_2O)	Sigma-Aldrich	756822
TRIS	VWR	0826
Other		
Cell Culture Dish (150x25 mm)	Corning	430599
25 mm Syringe Filter w/0.2 μm Polyethersulfone membrane	VWR	514–0072
15 mL Centrifuge Tube	Corning	430790
50 mL Centrifuge Tube	Corning	430828
Polypropylene Centrifuge Tubes (25x89 mm)	Beckman Coulter	326823
Polycarbonate Bottle with Cap Assembly (16x76 mm)	Beckman Coulter	355603
Nalgene Oak Ridge High-Speed Polycarbonate Centrifuge Tubes	ThermoFisher	3118-0085PK
Centrifuge 5810 R	Eppendorf	5811000015

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
WX+ Ultracentrifuge Series	ThermoFisher	75000080
Mega Star 3.0 R Centrifuge	VWR	521–1752
TX-750 Swinging Bucket Rotor	ThermoFisher	75003180
SW 32 Ti Swinging-Bucket Rotor	Beckman Coulter	369650
Type 70.1 Ti Fixed-Angle Titanium Rotor	Beckman Coulter	342184
F-34-6-38 Fixed-Angle Rotor	Eppendorf	EP5804727002

MATERIALS AND EQUIPMENT

PBS 10x

Reagent	Final concentration	Amount
KCl	27 mM	2 g
Na_2HPO_4	81 mM	14.4 g
KH_2PO_4	1,47 mM	2.4 g
NaCl	1370 mM	80 g
MiliQ Water	N/A	Up to 1 L
Total	N/A	1 L

Autoclave and store at room temperature (20°C) for up to 1 month.

Culture Media

Reagent	Final concentration	Amount
Fetal Bovine Serum	10%	50 mL
Penicillin/Streptomycin	1%	5 mL
Non-Essential Aminoacids	1%	5 mL
DMEM w/Glutamine	N/A	440 mL
Total	**N/A**	**500 mL**

Store at 4°C for up to 1 month.

ExoFree Culture Media

Reagent	Final concentration	Amount
ExoFree Fetal Bovine Serum	10%	50 mL
Penicillin/Streptomycin	1%	5 mL
Non-Essential Aminoacids	1%	5 mL
DMEM w/Glutamine	N/A	440 mL
Total	**N/A**	**500 mL**

Store at 4°C for up to 1 month.

Homogenization Buffer

Reagent	Final concentration	Amount
Sucrose	250 mM	1.71 g
EDTA 0.1 (pH 8)	1 mM	200 µL
Iodoacetamide	10 mM	20 µL
Phenylmethylsulfonyl fluoride 0.2 M	2 mM	200 µL
Cycloheximide 3 mM	0.03 mM	200 µL
Protease Cocktail Inhibitor 100x	1x	20 µL
Imidazole 3 mM (pH 7.4)	N/A	Up to 20 mL
Total	**N/A**	20 mL

Prepare the day before and store at 4°C.

Note: If required, protease inhibitors can be omitted from the homogenization buffer to maintain compatibility with downstream *in vitro* assays that measure proteolytic activity or require the addition of recombinant proteases. In such cases, keep all samples on ice and handle them at 4°C at all times to minimize unintended proteolysis.

25% (w/w) Sucrose Solution		
Reagent	Final concentration	Amount
Sucrose	25% (w/w)	2.76 g
Imidazole 3 mM (pH 7.4)	N/A	8.28 mL
Total	**N/A**	**10 mL**
Prepare the day before and store at 4°C.		

Note: To minimize microbial contamination, sucrose solutions are prepared using autoclaved glassware, pipettes and imidazole buffer and immediately stored at 4°C until use. To assess whether contamination is present, solutions should be visually inspected prior to use for turbidity/opalescence, precipitates, gas formation, or unexpected pH drift relative to freshly prepared buffer.

35% (w/w) Sucrose Solution		
Reagent	Final concentration	Amount
Sucrose	35% (w/w)	4.03 g
Imidazole 3 mM (pH 7.4)	N/A	7.43 mL
Total	**N/A**	**10 mL**
Prepare the day before and store at 4°C.		

Note: To minimize microbial contamination, sucrose solutions are prepared using autoclaved glassware, pipettes and imidazole buffer and immediately stored at 4°C until use. To assess whether contamination is present, solutions should be visually inspected prior to use for turbidity/opalescence, precipitates, gas formation, or unexpected pH drift relative to freshly prepared buffer.

62% (w/w) Sucrose Solution		
Reagent	Final concentration	Amount
Sucrose	62% (w/w)	8.05 g
Imidazole 3 mM (pH 7.4)	N/A	4.93 mL
Total	**N/A**	**10 mL**
Prepare the day before and store at 4°C.		

Note: To minimize microbial contamination, sucrose solutions are prepared using autoclaved glassware, pipettes and imidazole buffer and immediately stored at 4°C until use. To assess whether contamination is present, solutions should be visually inspected prior to use for turbidity/opalescence, precipitates, gas formation, or unexpected pH drift relative to freshly prepared buffer.

Tris/Sucrose/D_2O solution		
Reagent	Final concentration	Amount
Sucrose	30% (m/v)	30 g
Tris	0,2 M	2.4 g
D_2O	N/A	Up to 100 mL
Total	**N/A**	**100 mL**
Adjust pH to 7.4 and store at 4°C for up to 2 months.		

STEP-BY-STEP METHOD DETAILS

Isolation of small extracellular vesicles through differential ultracentrifugation

Timing: 4–5 h

This protocol isolates and purify sEVs, including exosomes, with sizes ranging from 30–150 nm.

1. Collect the culture media to a 50 mL sterile centrifuge tube (Ref.430828);
2. Balance the centrifuge tubes with PBS;
3. Centrifuge the culture media at 300 *g* for 10 min at 4°C in a TX-750 rotor, to remove dead cells;
4. Transfer the supernatant to a centrifuge tube (Ref.3118-0085PK);

⚠ CRITICAL: When decanting the tube, pour in a single smooth, continuous motion to avoid disturbing the pellet.

5. Balance the centrifuge tubes with PBS;
6. Centrifuge the remaining supernatant at 16500 *g* for 25 min at 4°C in a F-34-6-38 rotor.

Note: Ensure that the plastic tube used can withstand G-forces above 12,000 *g*.

7. Transfer the supernatant to a new 50 mL centrifuge tube (Ref.430828) and discard the pellet obtained in step 6;

Note: The fraction obtained in the pellet corresponds to microvesicles. If desired, it can be further purified with an additional PBS washing step under the same conditions as step 6.

8. Filter the supernatant obtained in step 6 through a 0.2 μm syringe filter, directly into a new ultracentrifuge tube (Ref.326823);
9. Fill the remainder volume of the tube with PBS up to 38 mL;
10. Balance the centrifuge tubes with PBS;
11. Centrifuge the filtrate at 120000 *g* for 70 min at 4°C in a SW 32 Ti rotor;
12. Discard the supernatant resulting from step 11;

⚠ CRITICAL: When decanting the tube, pour in a single smooth, continuous motion to avoid disturbing the pellet.

Note: Since the rotor used is a Swinging-Bucket, the pellet is expected to be evenly distributed at the bottom of the ultracentrifuge tube.

13. Add 38 mL of PBS to the pellet obtained in step 11, the sEVs fraction;

Note: In most situations the pellet will not be visible.

14. Centrifuge at 120000 *g* for 70 min at 4°C in a SW 32 Ti rotor, for a wash step;
15. Discard the supernatant resulting from step 14;

⚠ CRITICAL: After decanting the PBS from the ultracentrifuge tube, in a single smooth, continuous motion to avoid disturbing the pellet, maintain the tube inverted for the next step.

16. Dry the inside walls of the ultracentrifuge tube with absorbent paper, avoiding the bottom of the tube;

17. Resuspend the pellet from step 16 in 30 mL of cold PBS.
18. Add 4 mL of Tris/Sucrose/D_2O solution to a new ultracentrifuge tube (Ref.326823);
19. Gently add the sEVs resuspended in 30 mL of PBS from step 17 over the Tris/Sucrose/D_2O solution;

⚠ CRITICAL: PBS should be added as slowly as possible to not disturb the interface.

20. Centrifuge at 100000 *g* for 75 min at 4°C in a SW 32 Ti rotor;
21. With an 18-gauge needle, collect 3.5 mL of the Tris/Sucrose/D_2O solution, and add it to a new ultracentrifuge tube;
22. Add 35 mL of cold PBS to the ultracentrifuge tube;
23. Centrifuge at 120000 *g*, overnight (16 h), at 4°C in a SW 32 Ti rotor;
24. Discard the supernatant resulting from step 23.

⚠ CRITICAL: After decanting the PBS from the ultracentrifuge tube, in a single smooth, continuous motion to avoid disturbing the pellet, maintain the tube inverted for the next step.

Note: Since the rotor used is a Swinging-Bucket, the pellet is expected to be evenly distributed at the bottom of the ultracentrifuge tube.

25. Dry the inside walls of the ultracentrifuge tube with absorbent paper, without reaching the bottom of the tube;
26. Add appropriate volume of PBS or desired resuspension buffer directly to the bottom of the tube and scrape with the micropipette tip for 3 min;

⚠ CRITICAL: It is important to standardize scraping movements to maximize reproducibility.

27. Collect the sEVs into a microtube and store at −80°C;

Note: The integrity of the vesicles remains intact for up to 6 months.[7]

Endosome isolation through sucrose-density gradient ultracentrifugation

Timing: 2 days

This protocol allows the isolation of EE and LE with minimal contamination.

28. Wash the cells in ice-cold PBS three times;
29. Add 5 mL of ice-cold PBS, and with a cell scrapper, collect the cells to a 15–50 mL centrifuge tube (Ref.430790/430828), depending on the amount of cell culture dishes;

⚠ CRITICAL: Scraping must be performed very gently to prevent cell rupture.

Note: In this step, scraping can be replaced for trypsin-based assay.

30. Centrifuge the cells at 300 *g* for 5 min at 4°C and discard the supernatant;
31. Add 3 mL of the homogenization buffer described above to the pellet obtained in step 30, and gently resuspend with by mixing the solution in tube using a vortex-like motion with your hands;
32. Centrifuge at 1300 *g* for 10 min at 4°C in a TX-750 rotor and discard the supernatant;
33. Add 700 μL of homogenization buffer;
34. Gently hand vortex to dislodge the pellet;
35. Carefully transfer to a 2 mL microtube;

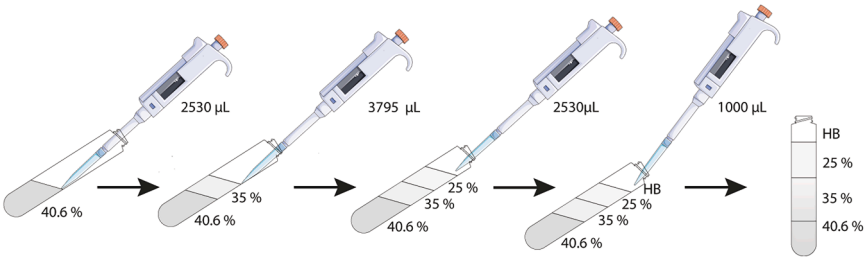


Figure 1. Workflow for discontinuous sucrose gradient layering

Representation of the layering of different sucrose solutions to form a discontinuous sucrose gradient from which early and late endosomes are separated.

36. Using a small glass pestle, previously chilled, resuspend the pellet with twenty strokes;

⚠ CRITICAL: Avoid the formation of bubbles, and check that there are no large aggregates.

37. With a 1 mL syringe, pass the suspension though a 25-gauge needle ten times;

⚠ CRITICAL: To avoid the formation of air bubbles, pre-rinse the syringe with homogenization buffer and avoid aspirating or expelling the full volume in a single motion.

Note: To confirm successful homogenization, place 5 µL of the suspension on a slide (with a coverslip) and examine it under a light microscope. Efficient homogenization is indicated by abundant intact nuclei and few, if any, intact cells.

38. Add 490 µL of homogenization buffer to the sample obtained from step 36, resulting in a solution made of 1 part homogenate to 0.7 parts of homogenization buffer.
39. Centrifuge at 1600 *g* for 10 min at 4°C in a TX-750 rotor, transfer the supernatant to a new 2 mL microtube and repeat the centrifugation, with the same conditions, to obtain the Post-Nuclear Supernatant (PNS);

Note: If intended, take a sample of the PNS in this step.

40. Transfer the PNS to a new 2 mL microcentrifuge tube and add homogenization buffer up to 1150 µL;
41. Add 1380 µL of 62% sucrose solution to the 1150 µL of PNS, resulting in a final sucrose concentration of 40.6%. Mix thoroughly until the solution is homogeneous, then transfer it to the bottom of an ultracentrifuge tube (Ref.355603);
42. Add 3795 µL of the 35% sucrose solution on top of the 40,6% sucrose solution (Figure 1);

⚠ CRITICAL: Pour gently to avoid mixing interfaces when overlaying the solutions.

43. Add 2530 µL of the 25% sucrose solution on top of the 35% sucrose solution (Figure 1);
44. Finally, add 1000 µL of homogenization buffer and balance the ultracentrifuge tubes (Figure 1);
45. Centrifuge at 210000 *g*, overnight (16 h), at 4°C in a 70.1Ti rotor;
46. In the next day collect the fractions (Figure 2):
    a. LE: Collect the top 2500 µL, including the homogenization buffer/25% sucrose interface;
    b. EE: Collect 2500 µL around the 25%/35% sucrose interface;

⚠ CRITICAL: Always collect the same volume to increase reproducibility.

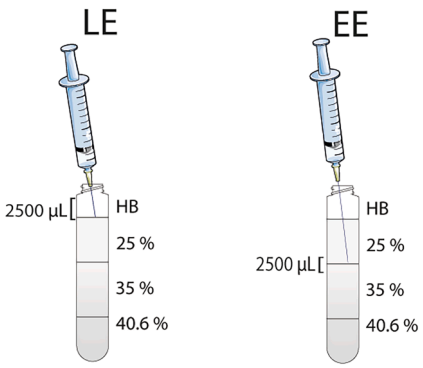


Figure 2. Early and late endosome recovery
Representation of the early endosome (EE) and late endosome (LE) fraction positions within the discontinuous sucrose gradient and their collection by syringe aspiration. LE are recovered from the homogenization buffer (HB) fraction including the HB and HB/25% sucrose interface, while EE are predominantly found between 25%/35% sucrose interface.

47. Add each sample to its own ultracentrifuge tube (Ref.326823) and fill it with 38 mL of 3 mM Imidazole solution;
48. Centrifuge at 100000 *g* for 1 h at 4°C in a SW 32 Ti rotor;
49. Discard the supernatants resulting from step 47;

⚠ CRITICAL: After decanting the PBS from the ultracentrifuge tube, in a single smooth, continuous motion to avoid disturbing the pellet, maintain the tube inverted for the next step.

Note: Since the rotor used is a Swinging-Bucket, the pellet is expected to be evenly distributed at the bottom of the ultracentrifuge tube.

50. Dry the inside walls of the ultracentrifuge tubes with absorbent paper, avoiding bottom of the tube;
51. Resuspend the pellets gently in an appropriate buffer and the desired volume. Subsequently quantify protein concentration of the isolated fractions;
52. Store the fractions in microtubes at −80°C.

Note: The integrity of the vesicles remains intact for up to 6 months.

EXPECTED OUTCOMES

Differential ultracentrifugation isolates a vesicle population with sizes ranging from 30 to 150 nm, enriched in exosomes and exosome markers such as CD63, Flotillin-1, Alix, and TSG101. For reference from $80x10^6$ ARPE-19 cells it is possible to obtain a protein yield between 2–5 µg for the sEVs fraction.

Furthermore, ultracentrifugation with the sucrose gradient separates EE and LE fractions by buoyant density. After overnight centrifugation, the EE fraction is expected to be between 25% and homogenization buffer interface. This fraction is enriched in markers such as EEA1 and Rab5 whereas LE fraction, recovered from the 25%–35% interface, is enriched in LAMP2 and Rab7. Moreover, minimal contamination between fractions should be observed when consistent volumes are collected. For reference, from $80x10^6$ ARPE-19 cells it is possible to obtain a protein yield between 40–60 µg for the LE fraction and 150–200 µg for the EE fraction.

Vesicle identities can be confirmed with Western Blot, Mass Spectrometry and Electron Microscopy that can reveal their contents, size and morphology. Nanoparticle Tracking Analysis and Flow

Cytometry in appropriate equipment can be used to confirm sEVs size and concentration. Acquired samples are suitable for proteomics, functional analysis and other assays.

Optimization may be necessary in terms of homogenization, as different cell types may require fewer or more passages through the syringe depending on their size. As a reference, ARPE-19 requires 10 strokes.

The protocol described herein has been implemented in peer-reviewed publications,[1,5] which may also serve as a quality control benchmark.

LIMITATIONS

The sucrose cushion approach can yield highly purified sEVs in a concentrated, low-volume fraction, reducing co-isolated protein corona and minimizing dilution. In contrast, size-exclusion chromatography and related methods often elute EVs across larger volumes, which can dilute samples and complicate downstream readouts such as Western blotting and proteomics. Manual steps, including cell homogenization, supernatant removal, and pellet resuspension influence the efficiency, since small variations in mechanical force during homogenization or scraping can lead to inconsistent release or recovery of vesicles. Similarly, incomplete removal of supernatants or inadvertent pellet disturbance during washing steps can result in variable vesicle loss between preparations. In addition, although sucrose gradient purification increases purity, it often results in reduced and more variable yields.

TROUBLESHOOTING

Problem 1

Lower vesicle yields than desired (step 26).

Potential solution

Increase number of cells in culture or/and increase cell incubation time in ExoFree media.

Problem 2

Lower EE and LE yields than desired (step 51).

Potential solution

Increase number of cells.

Problem 3

Collapsed centrifuge tube (steps 3,6,11,14, 19).

Potential solution

Ensure centrifuge tubes are balanced before centrifugation.

Problem 4

Disturbing the pellet after centrifugation (steps 3, 6, 11, 14).

Potential solution

Repeat the centrifugation to recover the pellet.

Problem 5

Air bubble formation during sample homogenization interferes with cell lysis due to shear stress disruption between the homogenate and the needle's wall (step 36).

Potential solution

Pre-rinse the syringe with homogenization buffer and avoid aspirating or expelling the full volume in a single motion.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, João Vasco Ferreira (joao.ferreira@nms.unl.pt).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contact, João Vasco Ferreira (joao.ferreira@nms.unl.pt).

Materials availability

This protocol did not generate new unique reagents.

Data and code availability

This protocol did not generate new data or code.

ACKNOWLEDGMENTS

This work was supported by the Research Unit UID/04462: iNOVA4Health - Programa de Medicina Translacional; the Associated Laboratory LS4FUTURE (LA/P/0087/2020); the TransNet Project (2022.08649.PTDC); and HERO Project (2024.17504.PEX), all financially supported by Fundação para a Ciência e Tecnologia/Ministério da Educação, Ciência e Inovação. This work was also supported by the European Union, 1181 EVCA Twining Project 1182 (Horizon GA n° 101079264). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

M.N.D. and L.P. wrote the draft and prepared the images. J.V.F. developed the protocol. J.V.F. and P.P. conceived the initial idea of the study. All authors reviewed and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Ferreira, J.V., Ferraz, L.C., da Rosa Soares, A., Ejtehadifar, M., Carvalho, A.S., Hall, M.J., Morello, J., Beck, H.C., Ramalho, J.S., Matthiesen, R., and Pereira, P. (2026). LAMP2A regulates endosomal protein composition and membrane identity in exosome biogenesis. iScience *29*, 114305. https://doi.org/10.1016/j.isci.2025.114305.

2. de Araújo, M.E.G., Lamberti, G., and Huber, L.A. (2015). Homogenization of mammalian cells. Cold Spring Harb. Protoc. *2015*, 1009–1012. https://doi.org/10.1101/pdb.prot083436.

3. Lässer, C., Eldh, M., and Lötvall, J. (2012). Isolation and characterization of RNA-containing exosomes. J. Vis. Exp. 1–6. https://doi.org/10.3791/3037.

4. de Araújo, M.E.G., Lamberti, G., and Huber, L.A. (2015). Isolation of early and late endosomes by density gradient centrifugation. Cold Spring Harb. Protoc. *2015*, 1013–1016. https://doi.org/10.1101/pdb.prot083444.

5. Vasco Ferreira, J., da Rosa Soares, A., Ramalho, J., Máximo Carvalho, C., Helena Cardoso, M., Pintado, P., Sofia Carvalho, A., Christian Beck, H., Matthiesen, R., Zuzarte, M., et al. (2022). LAMP2A regulates the Loading of Proteins into Exosomes. Sci. Adv. *8*, eabm1140.

6. Théry, C., Amigorena, S., Raposo, G., and Clayton, A. (2006). Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. Curr. Protoc. Cell Biol. *30*. https://doi.org/10.1002/0471143030.cb0322s30.

7. Gelibter, S., Marostica, G., Mandelli, A., Siciliani, S., Podini, P., Finardi, A., and Furlan, R. (2022). The impact of storage on extracellular vesicles: A systematic study. J. Extracell. Vesicles *11*, e12162. https://doi.org/10.1002/jev2.12162.