Superspeed centrifuges

Streamlining exosome and extracellular vesicle isolation with the LYNX 6000 and T29-8x50 integration – A comprehensive guide for researchers

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Introduction

Extracellular vesicles, including exosomes, are small membrane-bound vesicles released by cells that play a significant role in intercellular communication. They contain a diverse cargo of proteins, nucleic acids, and lipids, providing valuable information about the originating cells' physiological and pathological conditions. Isolating exosomes and other extracellular vesicles from complex biological samples can be challenging due to their low abundance and similarities to other vesicles.

Thermo Scientific™ Sorvall™ LYNX 6000 Centrifuge incorporated with Thermo Scientific™ T29-8x50 Fixed Angle Rotor simplifies the process of isolation, allowing researchers to perform all centrifugation steps in one unit. This application note provides a protocol for efficient isolation of exosomes and extracellular vesicles.

Figure 1 Sorvall LYNX 6000 Superspeed Centrifuge and T29-8x50 Fixed Angle Rotor

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Collecting culture supernatants for exosome purification

Collect and clear conditioned medium

1. Cultivate cells of interest under normal conditions until they reach 70% to 80% confluency for adherent cells or 60% to 70% of their maximum concentration for cells grown in suspension. [1]
2. Use an adequate number of cells to produce at least 70 ml of conditioned medium.[1]
    - » It is recommended to purify exosomes from larger volumes of conditioned medium as the yield of the purification process increases with the starting volume. This may require a minimum of seven 10-cm dishes or up to twenty 15-cm dishes.[1]
3. For adherent cells, remove the culture medium and replace it with an equivalent volume of exosome-production medium.[1]
4. For cells in suspension, centrifuge the cells for 10 minutes at 300 x g and 4°C. Pour off the supernatant and resuspend the cells in the same volume of exosome-production medium.[1]
5. Return the cells to the incubator for 24 to 48 hours. [1]
6. If the cells grow rapidly and become overconfluent, resulting in cell death after 48 hours, collect the conditioned medium after 24 hours.
    - » Alternatively, if the cells are highly sensitive and do not thrive in exosome-production medium or FBS-free medium, collect the supernatant after 24 hours.
7. For adherent cells: Collect the conditioned medium with a pipe and transfer to 50-ml polypropylene centrifuge tubes and centrifuge for 10 min at 300 x g at 4°C and collect the conditioned medium with a pipet.[1]
8. For cells in suspension: Centrifuge cells for 10 min at 300 x g at 4°C, collect the conditioned medium with a pipet and transfer to 50-ml polypropylene centrifuge tubes.[1]

Cellular debris removal

9. Centrifuge the cleared, conditioned medium for 10 min at 2,000 x g, 4°C. [2-9]
10. Transfer the supernatant to a highspeed tube and centrifuge for 30 min at 20,000 x g, 4°C. [2-9]

Exosome isolation

11. Transfer the supernatant from step 10 to fresh 50 ml PC Oak ridge tube. [2-8]
12. Ensure that none of the pellets are collected and contaminates the supernatant.
    - » In fixed-angle rotors, the pellet is on the side of the tube facing up, near the bottom. This side should be marked with a marker pen.
    - » When removing the supernatant using a pipette, tilt the tube at an angle to ensure that the pellet always remains covered with supernatant.
    - » Stop removing the supernatant when there is still approximately half a centimeter of liquid covering the pellet.
13. Run the samples at 29,000 rpm (100,605 x g) at 4 °C for 70 mins with T29-8x50 rotor in LYNX 6000 superspeed centrifuge. [2-9]
    - » For this high-speed centrifugation, all tubes should be at least 80% full. If one of the tubes is not three-quarters full, add PBS.
    - » For centrifugation at 4°C, the centrifuge loaded with T29-8x50 should be precooled before loading samples. [1-9]
14. Remove the supernatant completely.

Concentrating and washing exosomes

15. Resuspend the pellet in each tube in 1 ml sterile PBS [1]
    - » Combine the resuspended pellets from all tubes containing materials from the same cells into a single centrifuge tube.
    - » Fill the tube completely with PBS. It is likely that there won't be a visible pellet at this stage.
    - » Resuspend the contents by flushing up and down in the area where the pellet should be.
16. Repeat step 15, centrifuge resuspended pellets into PBS for 70 minutes at 100,605 ×g (29,000 rpm) at 4°C. [1-9]
17. Remove the supernatant as completely as possible.
18. Resuspend the exosome pellets into 50 to 100 µl of sterile PBS or TBS.
19. Store exosomes up to 1 year at –80 °C in 100-µl aliquots.[1-9]
    - » Repeated freezing and thawing must be avoided.

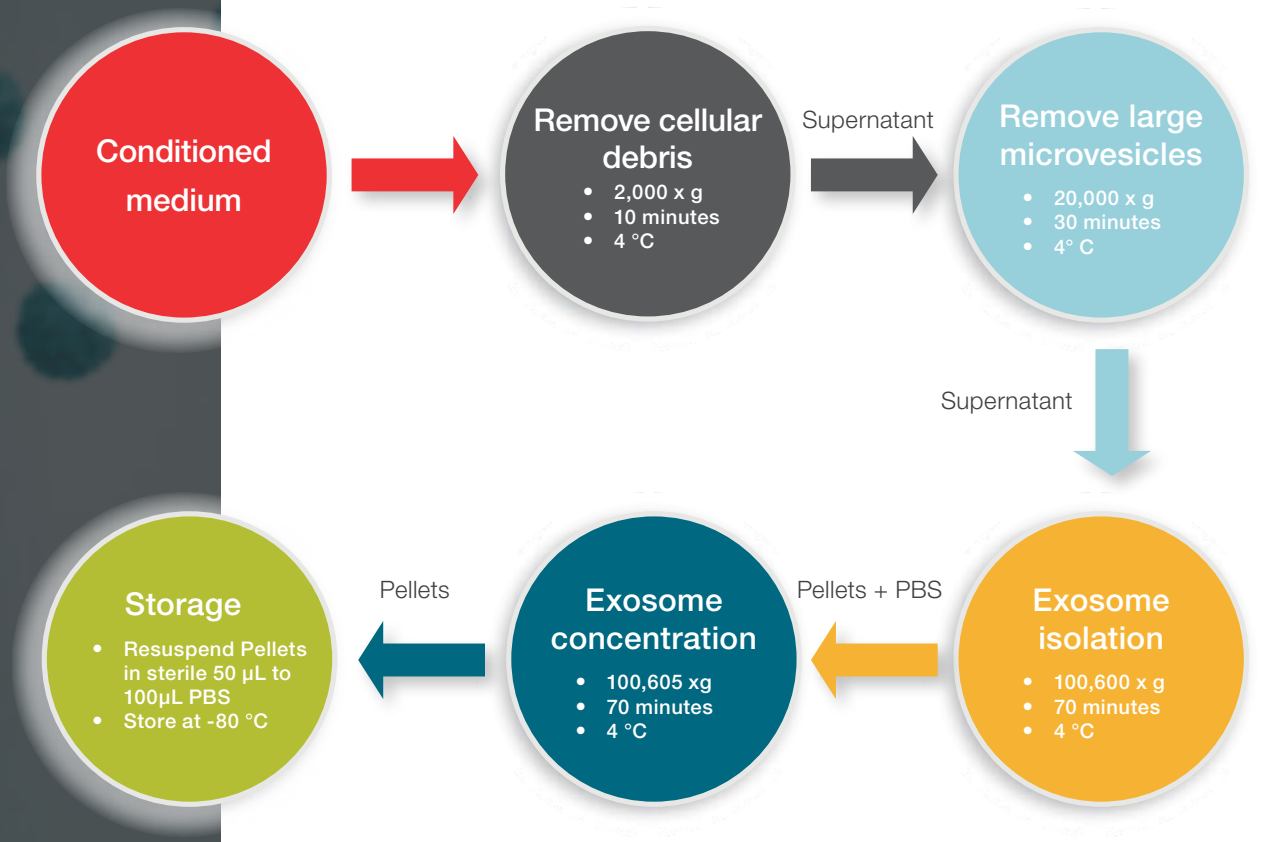

Figure 2 Exosome isolation protocol

Results

Characterization of isolated Evs from periodontal ligament stem cells (PDLSCs) using NTA demonstrated that isolated Evs distributed within the size range of 112 to 182 nm, as depicted in Figure 3C. The EVs preparations exhibited enrichment of exosomal markers CD63 and MFG-E8, as shown in Figure 3A. Furthermore, examination of EVs using transmission electron microscopy revealed the characteristic cup-shaped appearance of most particles, confirming the purity of the EV preparations, as illustrated in Figure 3B. [2]

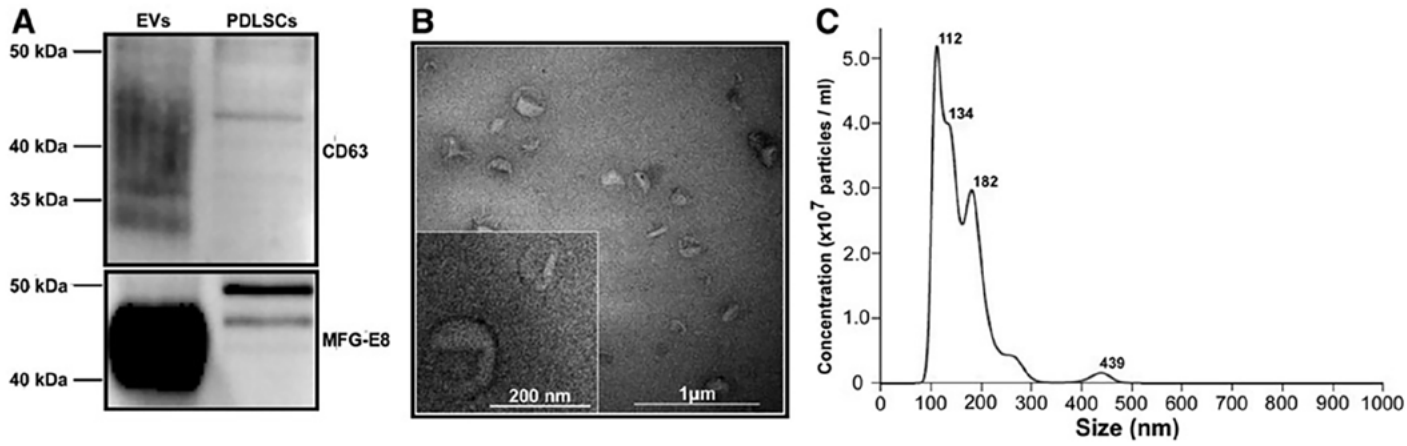

Figure 3 Characterization results of isolated EVs from periodontal ligament stem cells (PDLSCs) results by LYNX 6000 and T29-8x50 rotor adapted from "Extracellular Vesicles Suppress Basal and Lipopolysaccharide-Induced NFκB Activity in Human Periodontal Ligament Stem Cells" by Čebatariūnienė A, Kriaučiūnaitė K, Prunskaitė J, Tunaitis V, Pivoriūnas, Stem Cells Dev. 2019 Aug 1;28(15):1037-1049.

Conclusion

The integration of the T29-8x50 rotor with the LYNX 6000 centrifuge significantly simplifies the exosome isolation process, making it more accessible and cost-effective for research laboratories. While the preferred method for this type of application is to implement differential followed by density gradient using an ultracentrifugation platform, there are certain circumstances where access to an ultracentrifuge unit may be limited or there may be budget constraints to acquire a new unit. By following the protocol outlined in this application note, high-quality exosomes can be obtained using the LYNX 6000 centrifuge and T29-8x50 rotor for further studies.

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