ExoPLANT-**Lo**

Exosome Isolation from **Low** Water Content Plant Material Kit

Description

The ExoPLANT-Lo Kit provides a simple and effective method of concentrating intact exosomes from plant material containing low amounts of water (not juicy) or no water at all. The plant material suitable for exosome isolation with ExoPLANT-Lo could be fresh, dried, freeze-dried, or frozen. However, it is not possible to isolate intact exosomes from plant extracts or other preparations that might destroy membranous structures. The Kit is based on stabilization, precipitation and purification of exosomes using low-speed centrifugation.

Content

Components	Amount	Storage
Stabilizing Agent A	10 g	2°C to 8°C
Exosome Release Solution B (sterile)	200 mL	2°C to 8°C
Exosome Collecting Agent C (sterile)	100 mL	2°C to 8°C

Required but not Supplied

Equipment needed for the procedure and materials required but not present in the Kit:

- Centrifuge
- Weighing scale
- Fridge
- Centrifuge tubes
- Sterile 0.22 µm pore size filter*
- Disposable syringe for filtering
- Mortar and pestle
- Phosphate-buffered saline
- Automatic pipettes and tips
- Serological pipettes or measuring cylinders
- Liquid nitrogen**

** Low protein binding membrane filters are recommended for higher exosome yield.*

*** Only needed for the case of fresh material that is difficult to grind with a pestle.*

Process

SAMPLE PREPARATION

1. Weigh the plant material.
2. Transfer the plant material to a mortar.

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3. Use pestle to crush up the material into a powder. If the plant material is difficult to grind, add small amount of liquid nitrogen into the mortar and continue grinding.
   Note: take all safety measures required when working with liquid nitrogen.
4. Calculate the required amount of Stabilizing Agent A and Exosome Release Solution B corresponding to the amount of your plant material according to the ratio provided in the Table:

Plant material, g	Stabilizing Agent A, g	Exosome Release Solution B, mL
1	0,5	10

5. Add the Stabilizing Agent A and pour the calculated volume of Exosome Release Solution B into the mortar with plant material.
6. Use pestle to thoroughly mix the sample.
7. Transfer the mixture to a centrifuge tube.
8. Apply differential centrifugation to remove the pulp:
   8.1. centrifuge the mixture at 1000 × g for 5 minutes at 2°C to 8°C;
   8.2. collect the supernatant to a new centrifuge tube;
   8.3. centrifuge the supernatant at 10 000 × g for 10 minutes at 2°C to 8°C;
   8.4. collect the supernatant to a new centrifuge tube;
   8.5. repeat supernatant centrifugation until the pulp is removed and the supernatant becomes clear.

EXOSOME ISOLATION

9. Filter the supernatant through sterile 0.22 µm pore size filter.
10. Measure the volume of the filtered supernatant with a sterile serological pipette or measuring cylinder.
11. Add Exosome Collecting Agent to your filtered supernatant at the volume ratio 1:1.
    Note: the reagent is viscous. Pipetting must be done very slowly to prevent air gaps.
12. Mix well until solution becomes homogenous.
13. Incubate the mixture at 2°C to 8°C overnight or not less than 10 hours.
14. Centrifuge the mixture at 3000 × g for 60 minutes at 2°C to 8°C.
15. Discard all supernatant.
16. Resuspend the exosomes in phosphate-buffered saline or similar buffer.
    Note: exosomes are most stable when stored at plant-specific pH.
17. You may store isolated exosomes up to 3 months at -20 °C and up to 6 months at -80 °C.

EXOSOME QUALITY CONTROL

Exosome can be further analyzed by:
protein content | nucleic acid content | particle size and number | morphology and structure by TEM

For questions and troubleshooting support email info@exolitus.com

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