ExoPLANT-Hi

Exosome Isolation from High Water Content Plant Material Kit

Description

The ExoPLANT-Hi Kit provides a simple and effective method of concentrating intact exosomes from fresh plant material containing high amounts of water, such as juicy fruits, leaves, roots or other parts that can be easily juiced. Note that you can isolate exosomes only from fresh juice but not from stored ones. Also, 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 Collecting Agent B (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
- Fridge
- Centrifuge tubes
- Sterile 0.22 µm pore size filter*
- Disposable syringe for filtering
- Phosphate-buffered saline
- Automatic pipettes and tips
- Serological pipettes or measuring cylinders

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

Process

SAMPLE PREPARATION

1. Freshly prepare juice from your plant material.
2. Measure the volume of the juice.
3. Transfer the juice from plant material to a centrifuge tube.

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4. Add the appropriate amount of Stabilizing Agent A. For each 10 mL of juice, you will need 0.5 g of Stabilizing Agent A.
5. Mix well by vortex.
6. Apply differential centrifugation to remove the pulp:
   6.1. centrifuge the mixture at 1000 × g for 5 minutes at 2°C to 8°C;
   6.2. collect the supernatant to a new centrifuge tube;
   6.3. centrifuge the supernatant at 10 000 × g for 10 minutes at 2°C to 8°C;
   6.4. collect the supernatant to a new centrifuge tube;
   6.5. repeat supernatant centrifugation until the pulp is removed and the supernatant becomes clear.

EXOSOME ISOLATION

7. Filter the supernatant through sterile 0.22 µm pore size filter.
8. Measure the volume of the filtered supernatant with a sterile serological pipette or measuring cylinder.
9. Add Exosome Collecting Agent B 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.
10. Mix well until the solution becomes homogenous.
11. Incubate the mixture at 2°C to 8°C overnight or not less than 10 hours.
12. Centrifuge the mixture at 3000 × g for 60 minutes at 2°C to 8°C.
13. Discard all supernatant.
14. 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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