Plant Vesicle Extraction and Purification Kit (Dry Plant)

Cat #: BGT-OPU-09008

Size: 10T

Product Introduction

Plant-derived vesicles are rich in bioactive substances such as proteins, lipids, and RNA. Acting as natural signaling molecules and cargo transporters, they show great research and application potential in intercellular communication, immune regulation, and as drug delivery systems. However, their extraction faces technical bottlenecks due to the complex composition of plant tissues (interference from substances like polysaccharides, starch, pectin, etc.) and dense cell walls, leading to low yield and insufficient purity.

Addressing the characteristics of dry plant materials, Biogradetech has independently developed this extraction kit, which efficiently releases vesicles from plant tissues and effectively removes non-vesicle components. The extracted vesicles are suitable for electron microscopy analysis, NTA size analysis, nucleic acid analysis, protein analysis, cytological experiments, and animal experiments.

Materials Supplied and Storage Conditions

Kit components	Size（10T）	Storage conditions
Solution A	2.5 mL	-20°C
Solution B	60 mL	RT
1% Citric Acid	10 mL	RT
1M NaOH	25 mL	RT
0.45μm Filter	10 T	RT
EPF Purification Column	10 T	RT

Materials Required but Not Supplied

· High-speed centrifuge (capable of reaching 4°C and a centrifugal force of 10,000×g)

· Vortex mixer

· Electronic balance

· Pipettes

· Water bath

· 50 mL centrifuge tubes

· 1.5 mL centrifuge tubes

· 1×PBS buffer (sterile)

· pH test strips: 1-10 (wide range), 5.5-8.5 (precision)

· Fruit knife/scissors, beaker, cutting board, juicer/mortar, medical gauze, funnel, etc. (tools for juicing/grinding plants into homogenate)

Storage

Solution A is a brown suspension. Store at −20°C. Thaw at 4°C before use. It is recommended to aliquot to avoid repeated freeze-thaw cycles. Valid for 3 months at 4°C and 6 months at −20°C.

Other components can be stored at room temperature with a shelf life of one year. This kit is shipped with ice packs.

Procedure

1. Pre-Juicing Preparation

1.1 Juicer/Mortar Sterilization: Disassemble the juicer/mortar parts, spray with 75% alcohol and wipe carefully, then place in a biosafety cabinet for UV sterilization for at least 20 minutes.

1.2 Sample Preparation: Prepare the sample for extraction according to the kit's processing capacity.

Size（10T）	Centrifuged & Filtered Sample Volume
1 T	20 mL
10 T	200 mL

1.3 Sample Sterilization: Pour the dry plant material to be extracted into a beaker, rinse surface dust with 1×PBS,

remove the rinsed samples and place them in a new beaker, then place in a biosafety cabinet for UV sterilization for at least 20 minutes.

1.4 Sample Rehydration: Add an appropriate amount of 1×PBS to the container with the sample, ensuring the PBS submerges the sample. After the sample is rehydrated, remove the rehydrated sample and collect the rehydration water.

Note: Rehydration time varies significantly between different samples. It can be determined based on the characteristics and volume of the sample to be extracted. For reference, dry flowers require shorter rehydration time, while seeds and dry rhizomes require longer rehydration time.

2. Juicing/Grinding

2.1 Juicer Preparation: Under the biosafety cabinet, assemble the sterilized juicer parts according to the juicer's manual.

Note: If using a mortar, proceed directly to the next step.

2.2 Juicing/Grinding: Cut or snip the rehydrated and removed plant material into small pieces. Place the small pieces into the juicer/mortar, add an appropriate amount of rehydration water, and juice/grind until the plant juice separates from the residue and is fully released from the plant cells. Collect the juice to complete juicing.

2.3 Filtration for Debris Removal: Fold medical gauze into 8 layers and place it on a funnel. Pour the plant juice into the funnel. The collected filtrate is the coarse-filtered sample.

Notes:

a. To control endotoxins, perform juicing/grinding and related processes within a biosafety cabinet.

b. The juicer generates heat during operation, so juicing time needs to be controlled.

c. If using a mortar, the plant material can be ground first before adding rehydration water.

3. Centrifugation for Debris Removal

3.1 Low-Speed Centrifugation for Debris Removal: Centrifuge the coarse-filtered juice at 4°C, 5,000×g, for 10min. Collect the supernatant.

3.2 High-Speed Centrifugation for Debris Removal: Centrifuge the supernatant collected from step 3.1 at 4°C, 10,000×g, for 10min. Collect the supernatant.

4. Vesicle Extraction

4.1 Supernatant Pretreatment: Add well-mixed Solution A reagent to the plant juice after debris removal. The dosage is

as follows (other volumes can be scaled proportionally):

Centrifuged & Filtered Sample Volume: 20 mL; Solution A Volume: 200μL.

Note: Solution A must be mixed well before use.

4.2 Mixing and Incubation: After adding Solution A, cap the tube and invert to mix 8~10 times. Incubate the mixture in a 37°C water bath for about 8h or at room temperature for about 16h (overnight).

4.3 pH Adjustment: Take out the incubated mixture. Under a biosafety cabinet, adjust the pH of the mixture to 7.0 using the provided 1M NaOH or 1% Citric Acid.

4.4 High-Speed Centrifugation for Debris Removal: Centrifuge the pH-adjusted supernatant at 4°C, 10,000×g, for 20min. Collect the supernatant.

4.5 Filtration: Filter the collected supernatant through a 0.45μm filter. Finally, use a syringe to draw air and squeeze the liquid inside the filter to reduce sample loss.

4.6 Adding Extraction Reagent: Add Solution B reagent to the centrifuged and filtered supernatant obtained above. The dosage is as follows (other volumes can be scaled proportionally):

Centrifuged & Filtered Sample Volume: 20 mL; Solution B Volume: 5 mL.

4.7 Secondary Mixing and Incubation: After adding Solution B, tighten the tube cap and mix thoroughly using a vortex mixer for 1 minute. Let the mixture stand at 4°C for about 4 hours.

4.8 Precipitating Plant Vesicles: Centrifuge the incubated mixture at 4°C, 10,000×g, for 60min. Discard the supernatant.

4.9 Recentrifugation: Recentrifuge the tube containing the pellet at 4°C, 2,000×g, for 2min. Then, carefully aspirate and remove all supernatant.

Note: The pellet is rich in plant vesicles. Aspirate the supernatant as completely as possible.

4.10 Plant Vesicle Resuspension: Add a suitable amount of 1×PBS to the pellet and pipette gently and uniformly to resuspend. After dissolution, transfer the resuspension to a new 1.5mL EP tube (It is recommended to use about 400μL of 1×PBS to resuspend vesicles derived from every 20mL of plant juice).

4.11 Plant Vesicle Harvest: Centrifuge the 1.5mL EP tube containing the resuspension at 4°C, 10,000×g for 2min. Retain the supernatant, which is rich in plant vesicle particles.

Note: If a significant pellet remains, centrifuge multiple times at 10,000×g, 2min until no obvious pellet is visible,

collecting the supernatant each time.

5. Plant Vesicle Purification and Storage

5.1 Plant Vesicle Purification: Transfer the harvested crude plant vesicle particles into the upper chamber of the Exosome Purification Filter (EPF Column). Centrifuge at 4°C, 3,000×g, for 10min. After centrifugation, collect the liquid at the bottom of the EPF Column tube. This liquid is the purified plant vesicle particles. (If liquid remains in the upper chamber after centrifugation, repeat centrifugation until all liquid passes to the bottom of the EPF tube).

Note: The EPF Column is for single use only and should not be reused.

5.2 Plant Vesicle Storage: Aliquot the purified plant vesicles into appropriate volumes and store frozen at −80°C for subsequent experiments.

Notes

1. For the Solution A, aliquot according to single-use volumes upon receipt and store at −20°C. Thaw before use.
2. Solution A may form a brownish-yellow precipitate upon standing, which is normal. Mix well before use.
3. Purified plant vesicles can be stored in a −80°C freezer. Avoid repeated freeze-thaw cycles to prevent vesicle rupture.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.