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Purification of Plant Extracellular Vesicles

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol outlines a method for the purification of viral particles and plant extracellular vesicles using polyethylene glycol (PEG) precipitation. The protocol is optimized for selective concentration of viral and vesicular components from plant samples while minimizing contaminants. The expected results include a purified preparation of viral particles and plant-derived vesicles that are suitable for downstream analysis, such as electron microscopy or molecular characterization. This version of the protocol incorporates optimizations to enhance the yield and quality of the purified particles, improving their stability and integrity during analysis.

Guidelines

- Ensure that all components, including PEG 6000, NaCl, and Tris-HCl (pH 7.5), are properly weighed and dissolved to prepare the stock solution.
- Use fresh extraction lysate and immediately mix with the stock solution to avoid degradation of viral particles and extracellular vesicles.
- Maintain proper ratios: add 1 part of stock solution to 2 parts of extraction lysate for optimal viral and vesicle precipitation.
- Use protease inhibitors to preserve the integrity of proteins during viral particle purification.



Materials

Materials:

- **PEG 6000 (Polyethylene Glycol 6000):** Precipitation of viral particles and extracellular vesicles.
- **NaCl (Sodium Chloride, 1.5 M):** Maintains isotonic conditions during precipitation.
- **Tris-HCl Buffer (pH 7.5, 1 M):** Ensures proper pH for the process.
- **Pierce Protease Inhibitor Tablets:** Protects viral particles and vesicles from protein degradation.
- **PES Filter Material (0.45 µm):** Removes large debris.
- **Centrifuge:** Required for multiple centrifugation steps.
- **50 mL Tubes (Falcon):** For sample processing and storage.
- **Homogenizer (Hand Model with Ceramic Balls):** Used for smashing plant material.
- **Extraction Bags:** Sterile bags for homogenization.
- **Deionized Water**
- **Parafilm**
- **Ice Bath**

Buffers:Extraction Buffer (1 X):

- 50 mM Tris-HCl (pH 7.5)
- 50 mM NaCl
- 1 Pierce Protease Inhibitor Tablet per 50 mL
- Keep cold on ice.

Precipitation Buffer (3 X):

- 36% PEG 6000 (w/v)
- 1.5 M NaCl
- 150 mM Tris-HCl (pH 7.5)

Safety warnings

- ! ▪ Handle PEG 6000 with care to ensure accurate concentrations, as incorrect amounts could affect the purification efficiency.
- Ensure that NaCl is fully dissolved to avoid uneven distribution in the purification mixture.
- Protease inhibitors should be added immediately before use to ensure that they remain effective throughout the purification process.
- Proper waste disposal is required for PEG and any biological materials according to institutional safety guidelines.



Before start

- Prepare all stock solutions (PEG 6000, NaCl, Tris-HCl) in advance to ensure smooth execution of the protocol.
- Ensure that the extraction lysate is fresh and properly prepared to avoid any degradation of viral particles or vesicles.
- Check the pH of Tris-HCl buffer and adjust if necessary to maintain consistency.
- Have protease inhibitors ready before beginning the purification process to preserve proteins.

Sample Collection

1 Collect Samples (20 grams):

- **Petioles:** Carefully cut the petioles from the base where the leaf connects to the stem (15 grams).
- **Twigs:** Use clean, sterilized scissors or a scalpel to cut small sections of twigs. Ensure the samples are taken from healthy or symptomatic regions, depending on your research focus (5 grams).

Sample Homogenization

2 Prepare Cold Buffer:

- Combine 50 mL of 50 mM Tris-HCl (pH 7.5) and 50 mM NaCl.
- Add 1 Pierce Protease Inhibitor Tablet per 50 mL.

1. Smash Plant Material:

- Place plant material in a sterile extraction bag.
- Add cold buffer to the bag.
- Homogenize using a **Bioreba hand model homogenizer with ceramic balls** until fully smashed.



Bioreba hand model homogenizer

Centrifugation and Filtration:**Centrifuge:** Transfer homogenate to a clean 50 mL Falcon tube and centrifuge at **8000 × g for 10 minutes** to remove plant debris.

Filtration: Filter the supernatant through a **0.45 µm PES sterile filter** to remove large particles.



PEG Precipitation

3 Prepare Precipitation Mixture:

- Add 1 part of **precipitation buffer (3 X)** to 2 parts of the filtered lysate.
- Final concentrations achieved:
- PEG 6000: **12% (w/v)**
- NaCl: **0.5 M**
- Tris-HCl (pH 7.5): **50 mM**

Incubate: Keep the mixture at **4°C overnight** for precipitation.

Centrifugation and Pellet Washing

4 **Centrifuge:** Spin the sample at **10,000 × g** for **1.5 hours** at 4°C.

Aspirate Supernatant: Carefully remove the supernatant without disturbing the pellet.

Wash Pellet (2x):

- Wash the pellet with **10 mM Tris-HCl buffer (pH 7.5)** twice to remove residual PEG and salts.

Suspend Pellet:

- Suspend the pellet in **10 mM Tris-HCl buffer (pH 7.5)** for use in enzymatic experiments and nucleic acid extractions.



Protocol references

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2. Alexander, Marina R., Christina L. Rootes, Petrus Jansen van Vuren, and Cameron R. Stewart. "Concentration of infectious SARS-CoV-2 by polyethylene glycol precipitation." *Journal of Virological Methods* 286 (2020): 113977.