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Protocol for Proteome Extraction from Norway Spruce Bark Samples

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We use this protocol and it's working

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Abstract

Proteomic analysis of Norway spruce bark presents unique challenges due to the high content of non-protein contaminants like phenolic compounds and polysaccharides. The use of more conventional methods such as TCA-acetone precipitation often falls short in such contexts. Here we present an optimized protocol leveraging TRIzol™ reagent in combination with dithiothreitol (DTT) and polyvinylpolypyrrolidone (PVPP). While DTT helps maintain proteins in their reduced state, preventing aggregation, PVPP effectively sequesters phenolic compounds, preserving protein integrity. This synergistic approach increases protein yield and purity, overcoming the limitations of conventional methods and providing a reliable solution for protein extraction from plant tissues.

Guidelines

The extraction of proteins from plant tissues such as the bark of Norway spruce presents significant challenges, mainly due to the presence of non-protein contaminants such as lipids, phenolic compounds, and polysaccharides. These compounds, especially phenolics, can strongly interact with proteins, leading to issues such as streaking and smearing in 2-D electrophoresis, and the formation of irreversible complexes that hinder any downstream analysis (Laing & Christeller, 2004; Yadav et al., 2020). Common procedures such as TCA–acetone precipitation, although effective in some plant tissues, did not yield satisfactory results for our specific samples. This led us to explore an alternative approach combining TRIzol™ reagent, DTT, and PVPP (Isaacson et al., 2006; Méchin et al., 2006; Zienkiewicz et al., 2014). TRIzol™ is a highly reliable reagent extensively used in molecular biology for efficient extraction of RNA, DNA, and proteins from various samples. Nonetheless, its effectiveness in extracting proteins from plant tissues, specifically those abundant in secondary metabolites like spruce bark, lacks comprehensive documentation. As a first step, DTT was incorporated into our protocol. DTT is a reducing agent known for its ability to prevent the formation of disulfide bonds between cysteine residues in proteins (Rabilloud, 1996). In the context of our extraction procedure, DTT helped to maintain proteins in their reduced state, thus preventing the formation of complex aggregates. This property is particularly relevant in the context of plant proteomics, where proteins may be more prone to form complexes due to the diverse range of enzymatic and structural proteins present in the tissues. The incorporation of PVPP into the extraction protocol is set to enhance both the yield and purity of the extracted proteins (Jiang et al., 2017). PVPP serves as a highly effective means of reducing the interference of phenolic compounds. These, which are abundant in plant tissues, are notorious for their strong affinity to proteins, leading to complex formations that can hinder protein solubilization and purification. The unique properties of PVPP, in particular its high surface area and strong binding affinity for phenolics, allow it to sequester these compounds (Laborde et al., 2006), providing reliable and reproducible samples for downstream proteomic analyses. Together, these components create a synergistic effect that enhances the efficiency and effectiveness of our protein extraction protocol, making it particularly well-suited for the extraction of proteins from more challenging plant tissues such as Norway spruce bark.

Materials

- IKA mill (IKA-Werke, Staufen, Germany)
- Ground Norway Spruce bark samples
- 2 ml microcentrifuge tubes
- Polyvinylpolypyrrolidone (PVPP) (Sigma Aldrich, Vienna, Austria)
- TRIzol™ reagent (Thermo Fisher Scientific, Vienna, Austria)
- Dithiothreitol (DTT) (Merck, Vienna, Austria)
- Chloroform (Merck, Vienna, Austria)
- Ethanol (100%) (Merck, Vienna, Austria)
- Isopropanol (Merck, Vienna, Austria)
- Wash solution (0.3 M guanidine hydrochloride (Sigma Aldrich, Vienna, Austria) in 95% ethanol)
- Urea buffer: 8M Urea (Merck, Vienna, Austria), 50mM Tris-HCl (Sigma Aldrich, Vienna, Austria), pH 8.0, , supplemented with 50 mM DTT and proteinase inhibitors

Sample Preparation

- 1 Start with frozen bark samples.
- 2 Use an IKA mill to grind the frozen bark samples into fine dust. It's important to keep the samples always frozen during the grinding process, although it is not necessary to use this specific mill.
- 3 Weigh 100 mg of the sample and transfer it into a 2 ml microcentrifuge tube.

Cell Lysis and Protein Extraction

- 4 Add 10 mg of PVPP to inhibit phenolic compounds.
- 5 Mix the sample with TRIzol™ reagent and DTT, then incubate for 5 minutes at room temperature.
- 6 Centrifuge the lysate at 12,000 × g for 5 minutes at 4°C.
- 7 Carefully transfer the clear supernatant to a new tube.

Phase Separation

- 8 Add 0.2 mL of chloroform per 1 mL of TRIzol™ reagent used. Shake vigorously to mix.
- 9 Allow the mixture to incubate for 5 minutes at room temperature.
- 10 Centrifuge at 12,000 × g for 15 minutes at 4°C.



- 11 Carefully transfer the organic phase, which contains the proteins, to a new tube. Avoid contamination from the aqueous phase.

Protein Precipitation

- 12 Add 0.3 mL of 100% ethanol per 1 mL of TRIzol™ reagent used.
- 13 Mix and incubate for 5 minutes at room temperature.
- 14 Centrifuge at 12,000 x g for 5 minutes at 4°C.
- 15 Transfer the supernatant to a new tube.
- 16 Add 1.5 mL of isopropanol per 1 mL of TRIzol™ reagent to the supernatant.
- 17 Incubate with end-over-end rotation overnight at 4°C.

Washing

- 18 Centrifuge at 12,000 x g for 10 minutes at 4°C and discard the supernatant.
- 19 Resuspend the pellet in 2 mL of wash solution. Incubate for 20 minutes at room temperature.
- 20 Centrifuge at 10,000 x g for 10 minutes at 4°C and discard the supernatant.
- 21 Repeat the washing steps twice for a thorough cleaning of the pellet.

Final Purification



- 22 Add 2 mL of 100% ethanol to the pellet and vortex to mix. Incubate for 20 minutes at room temperature.
- 23 Centrifuge at 10,000 x g for 10 minutes at 4°C.
- 24 Carefully remove and discard the supernatant.
- 25 Allow the pellet to air dry for 5 minutes. Avoid over-drying to the point of cracking.

Protein Solubilization

- 26 Resuspend the pellet in 50 µL of Urea buffer containing 50 mM DTT and proteinase inhibitors.
- 27 Allow the proteins to resuspend at room temperature for one hour. Then, incubate with end-over-end rotation overnight at 4°C.