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Sample Preparation of *Hevea brasiliensis* for Proteomic Analysis by LC-MS/MS

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of Hevea brasiliensis samples for quantitative proteomic analysis using the SP3 method (single-pot, solid-phase-enhanced sample preparation). The tissue samples are homogenised in SDS-containing lysis buffer, then sonicated, rotated and the proteins quantified. The proteins are reduced, alkylated and bound to paramagnetic SP3 beads and then enzymatically digested with trypsin/Lys-C. After peptide salting with SDB-based reversed-phase tips, the samples are dried, reconstituted and quantified prior to LC-MS/MS analysis. This workflow enables efficient and reproducible sample processing of complex plant tissues with minimal sample loss.

Protocol Steps

1 Cut the sample into small pieces using sterile scissors. Add lysis buffer containing 100 mM Tris-HCl (pH 8.0), 4% sodium dodecyl sulfate (SDS), and 20 mM NaCl.

2 Sonicate the sample using a sealed ultrasonic homogenizer for 30 minutes, then rotate at room temperature for 24 hours.

3 Repeat the sonication for 30 minutes and rotate again for 24 hours.

4 Sonicate once more for 30 minutes, then filter the lysate using a 0.22 µm membrane filter.

5 Measure protein concentration using the bicinchoninic acid (BCA) assay. Adjust the protein concentration to 0.15 µg/µL with the same lysis buffer (100 mM Tris, 4% SDS, and 20 mM NaCl).

6 To reduce disulfide bonds, add tris(2-carboxyethyl)phosphine (TCEP) to a final concentration of 20 mM and incubate at 80°C for 10 minutes.

7 To alkylate cysteine residues, add iodoacetamide (IAA) to a final concentration of 30 mM and incubate in the dark at room temperature for 30 minutes.

8 Prepare single-pot solid-phase-enhanced sample preparation (SP3) beads by mixing equal volumes of hydrophilic and hydrophobic Sera-Mag SpeedBeads (1:1 v/v), washing them three times with distilled water, and suspending them at 8 µg/µL in distilled water.

9 Add 20 µL of SP3 beads to the alkylated sample, then add three volumes of ethanol. Mix at room temperature for 20 minutes.

10 Wash the beads twice with 80% ethanol. Add 100 µL of 50 mM Tris-HCl (pH 8.0) and mix.

11 Digest the proteins by adding 200 ng of Trypsin/Lys-C Mix (Promega) and incubate overnight at 37°C.

12 Add 20 µL of 5% trifluoroacetic acid (TFA) and sonicate the sample.

13 Desalt the peptides using GL-Tip SDB (GL Sciences), then dry the eluate using a centrifugal evaporator.

14 Dissolve the dried peptides in 2% acetonitrile (ACN) and 0.1% TFA. Sonicate the solution to aid dissolution.

15 Measure peptide concentration with a BCA assay. Adjust the concentration to 200 ng/µL with 2% ACN and 0.1% TFA.

16 Proceed to liquid chromatography–mass spectrometry (LC-MS) analysis.