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Milled Wood Lignin Isolation for NMR

Supplies and Reagents:

Acetone
50 ml Glass tube
Cellulase (Sigma C9422-10 KU)
Acetate buffer (pH 4.5, 20 mM)
P_2O_5

Important Notes:

1. Dry the wood dust (40-60 mesh) and milled wood before process.
2. Insert the flat seal and make sure that the groove is situated in the area of one of the locking hooks.
3. Tight the actuating pin with a screw driver.

Protocol:

1. Wood Extraction: After harvest and scrap the developing xylem, put the wood into the glass tube, and add acetone immediately. After every 48 hours, change the acetone with acetone: water (90:10) for totally three times.
2. Dry the wood in the hood.
3. Use the Wiley Mini Mill (Green House) to mill the wood to 40-60 mesh.
4. Dry the wood dust.
5. Weight 2 g wood dust; put the wood dust and 17 balls into the bowl.
6. Set the bowls into the Planetary mill, set the speed at 600 rpm, milling time for 30 min, pause time for 15 min, turn on the reverse, and repeated the cycle for 6.
7. Collect the milled wood from the bowls.
8. Use 1 g milled wood and 450 unit of cellulase for the incubation.
9. Incubate the tube in 48℃ for 48 hours, at 180 rpm.
10. Centrifuge the tube and wash the cellulase treated milled wood with acetate buffer and Water twice.
11. Freeze dry the CEL (Cellulolytic Enzyme Lignin).
12. Weight the CEL.