

# Isolation of Microsomal Fraction from Plant Total-Protein Extracts

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## Materials and Supplies

- Optima MAX-XP Ultracentrifuge (Beckman Coulter)
- Beckman Coulter Rotor MLA-80
- Beckman Coulter Polycarbonate Thickwall Open-Top Tubes (#355647)
- Protein Suspension Apparatus
- Ice/Bucket

## Recipe

- **TES-TG** (50 mM Tris-HCL pH 7.5, 20% glycerol)

## Important Notes

- Always keep protein on ice. Never allow samples to reach room temperature or over-heat.
- Ultra-centrifuge must be pre-cooled to 4°C.
- Do not allow samples to dry. Add buffer immediately post microsome isolation.
- Glassware and centrifuge tubes should be pre-chilled on ice before the experiment.

## Protocol

1. Pre-cool the ultracentrifuge and rotor to 4°C.
2. Pre-chill Buffer TES-TG, centrifuge tubes and protein suspension apparatus on ice.
3. Carefully pipette 6ml of total protein into each ultracentrifuge tubes. Leave on ice.
4. Balance the ultracentrifuge tubes using pre-chilled TES-TG.
5. Centrifuge at 100,000 x g for 2 hours at 4°C.
6. After centrifugation, there should be a visible green pellet at the bottom. Pour off the supernatant, and gently wash the pellet twice with chilled TES-TG.
7. Resuspend the pellet in 1ml of chilled TES-TG using the protein suspension apparatus.
8. Aliquot and store at -80°C.