

Protocol for Fungal Biomass Water Depletion Stress

Fungal Biomass Preparation and Growth

All biomasses are grown on MEA (Malt Extract Agar) culture media by plating 150 ul of each strain suspension. To facilitate future transfer of biomasses in stress conditions, they have not been inoculated directly on MEA, but plated on a sterile cellophane membrane (BIORAD, <https://www.bio-rad.com/it-it/sku/1650963-cellophane-membrane-backing?ID=1650963>).

Biomasses are then incubated at their optimal conditions.

Preparation per litre MEA

- 1) Suspend in 1000 mL of distilled water
 - 30 g of Malt Extract
 - 15 g Bacteriological Agar
- 2) Mix with frequent agitation to completely dissolve all compounds.
- 3) Autoclave at 121°C for 20 minutes

Water Stress

The film coated by biomass is transferred on a self-made based Malt Extract Agar culture media to provide a water depletion stress.

The treatment time under stressing condition is 7 days at optimal temperature (depending on the species requirements). Biomasses are then ready for lyophilization and for RNA extraction.

Preparation for 1 L of water stress medium

- 1) Suspend in 1000 mL of purified water the following quantities of compounds:
 - 20 g of Malt Extract
 - 20 g Bacteriological Agar
 - 5 g of Yeast Extract
 - 100 g of NaCl
 - 120 g of D-glucose
- 2) Mix with frequent agitation to completely dissolve all compounds.
- 3) Autoclave at 121°C for 20 minutes.