

# Protocol for Oxidative and Oligotrophic 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 sterilized cellophane membrane (BIORAD, <https://www.bio-rad.com/it-it/sku/1650963-cellophane-membrane-backing?ID=1650963>).

Fungal strains are then incubated at their optimal temperature.

The film coated by biomass grown on MEA (Malt Extract Agar) at optimal condition are then incubated on a oxidative and oligotrophic stress- based media for one-week.

Biomasses are then ready for lyophilization and for RNA extraction.

## Oxidative and Oligotrophic Stress

The film coated by biomass is transferred on the culture medium for the specific stress condition.

A Czapek-based added with Menadione 1 mM culture media is used for oxidative and oligotrophic stress.

The treatment time in stress condition is 7 days at optimal temperature (according to the species).

## Preparation for 1 L of oxidative/oligotrophic medium

- 1) Suspend 49 g of Czapek Dox Agar in 1000 mL of purified water, then add 17,218 g of Menadione.
- 2) Mix with frequent agitation to completely dissolve the powder.
- 3) Autoclave at 121°C for 20 minutes.