

Isolation of spores with Zymolyase

Spores are released from the sporangia by enzymatic digestion of the hyphal cell walls with Zymolyase.

1. AFM plates are inoculated with spores in the middle of the plate. The plates are incubated for 5 to 10 days at 30 °C
2. The mycelium is stripped off the plate with a loop, a pipette tip or a glass slide and resuspended in 5 ml of sterile water in a 13 ml Sarstedt tube by shaking and vortexing.
If a very clean prep is required, it should be tried to take the top layer of mycelium, only (spores). Taking any agar-medium should be avoided.
3. 0.5 ml Zymolyase 20T solution (15 mg/ml) are added and the suspension incubated at 37 °C for 1 – 2 h until the hyphal cell wall is totally digested and the spores released.
It is important not to vortex the suspension after the enzyme has been added. Otherwise the Zymolyase might be destroyed.
4. 5 ml of sterile spore buffer (0.03 % v/v Triton X-100) are added and the spores are collected by centrifugation at 2000 rpm for 5 min (Hereaus Minifuge).
5. The spores are washed three times in spore buffer and finally resuspended in 1 ml spore buffer.

The isolated spores are kept at 4 °C for 2 weeks or can be stored in 20 % glycerol (400 µl 50 % glycerol + 600 µl spore prep) at –70 °C

Zymolyase stock (15 mg/ml)

- Dissolve 150 mg Zymolyase (kept at 4 °C) in 10 ml H₂O in a 15 ml Falcon tube.
- Filter it into a new tube by the use of a 0.22 µm syringe filter.
- Make 1.5 ml Aliquots and freeze at –20 °C