

Methods for assessment of oospore viability

Oospore germination

1. Lyse mycelial fragments and sporangia in the oospore suspension using the NovoZym treatment.
2. Spread oospores (approx. 1 ml of oospore suspension) on a 9 cm Petri plate containing 10-ml sloppy water agar (5 g.l⁻¹).
3. Tape the plates with Parafilm and incubated for 14 d at 20°C under cool blue fluorescent light.
4. Oospore germination can be assessed using a reversed microscope (at a magnification of 10 x 10).

Plasmolysis test

1. Extract oospores from agar plates as described.
2. Add an equal volume of 4 M NaCl to the oospore suspension and mix by inversion.
3. Incubate the suspension for 3 h at 20°C.
4. Plasmolysis can be assessed by microscopically observation. Viability is expressed as the percentage of oospores that were plasmolysed.

NOTE: In case of routine assessments of oospore viability, there is no need to include a Novozym treatment prior to the plasmolysis or MTT staining technique, as the NovoZym treatment generally will reduce oospore viability.

MTT viability staining technique

1. Extract oospores from agar plates as described.
2. Prepare a 0.1 M Phosphate buffer (pH 5.8) and add 0.1 % (0.1 g in 100 ml buffer) tetrazolium bromide (MTT), stir well.
3. Add an equal volume of MTT solution to an oospore suspension in a 50 ml disposable centrifuge tube, close the lid and mix well by inverting.
4. Incubate the oospore suspensions for 2 d at 35 °C.

5. Transfer a small aliquot (approx. 50 μ l) of the suspension to an object glass and examine the colour of individual oospores. Rose coloured oospores are considered to be dormant, blue-pink oospores are assumed to be activated (ready to germinate) and unstained or black oospores are considered to be non-viable.
6. Calculate the percentage viable oospores (dormant + activated) based on at least 250 examined oospores.

NOTE: Tetrazolium bromide is a extremely toxic compound, make sure to protect yourself from exposure to MTT and work according to your local safety regulations.