

## **Production and extraction of oospores from agar based media**

### **In vitro production of oospores on solid agar media**

1. Transfer isolates to be used as parents in crosses to Petri plates containing Rye agar (RA) amended with 0.05 g l<sup>-1</sup> β-sitosterol and incubate for 10-14 d.
2. Take two or three small agar plugs (5 mm diam) from the margin of the fast growing colony and place the plugs on one side of a 9 cm Petri plate containing RA amended with 0.10 g l<sup>-1</sup> β-sitosterol.
3. Place two or three agar plugs from the other parent on the other side of the plate, about 3 cm apart from the first parent.
4. Seal the plates with Parafilm and incubate in the dark at 15-20 °C for 14 d (after 7d check for oospore formation at the junction where the two parental colonies meet).

*NOTE: In literature, many agar media have been described that facilitate large numbers of oospores to be produced. We have encountered difficulties using V8 based media since a fairly large group of Mexican and western European isolates appears to grow slow on V8 Media. We have tested Pea Agar but found few oospores formed. The best advice is to try several media in your lab and select the one that performs best.*

### **Extraction of oospores from agar based media**

1. Mark the mating region (a distinct band visible by eye showing extensive stimulation of submerged hyphal growth at the interaction zone between the two parental strains) with a permanent marker on the bottom of the Petri dish.
2. Excise the mating region using a scalpel, transfer agar pieces into sterile 50 ml centrifuge ("blue cap") tubes containing 9 ml sterile tap water.
3. Place a homogeniser (we are using a IKA T20 homogeniser with S20 probe) in a laminar flow cabinet and thoroughly sterilize the probe by

30 s full speed mixing using a beaker containing 96% Ethanol. Rinse twice with tap water.

4. Blend the agar for 60 s at 20,000 rpm.
5. Quantify oospore concentration by counting oospores in three 50  $\mu$ l aliquots.

*NOTE: A NovoZym treatment can be applied to lyse any mycelial fragments and sporangia in the oospore suspension.*

6. Prepare a NovoZym 234 solution by adding 50 mg NovoZym 234 (Novo Biolabs) per ml sterile tap water.
7. Filter sterilize the NovoZym solution using a 0.2  $\mu$ m filter. Keep in refrigerator until use.
8. Add 1 ml of NovoZym solution to 9 ml of oospore suspension, incubate for 24 h at 20 °C.
9. Wash oospores in three successive steps by adding 25 ml sterile tapwater, spinning down the oospores using a tabletop centrifuge, carefully remove supernatant using a pipette and re-suspended in 10 ml sterile tap water.