Ashbya Clean Spore Prep

jlekena

Protocol Info: jlekena: Ashbya Clean Spore Prep. **protocols.io** https://protocols.io/view/ashbya-clean-spore-prep-b52cq8aw

Created: Mar 07, 2022

Last Modified: Mar 10, 2022

PROTOCOL integer ID: 59172

ABSTRACT

Preparation of purified A. gossypii spores that can be used for microscopic observations and for spore dissection. This protocol takes advantage of the hydrophobic surface of the spores to remove them from a mix of spores and hyphae.

GUIDELINES

Triton appears to have a negative effect on spore germination. For live cell microscopy, make sure most of the Triton is removed from the spores either by washing gently with media, or diluting to at least 1:1000 in culture.

MATERIALS

Sigmacote - Sigma Aldrich SL2-100ml
Screw cap test tube (DWK 73750-16150 or equivalent)
Black Phenolic screw cap (DWK 73800-15415 or equivalent)
37ºC incubator with tube rotator/roller (ideally)
sterile 50% glycerol
sterile 0.1% Triton X-100
sterile 0.03% Triton X-100
Screw cap tubes with o-rings for storing frozen spores (any brand will do)

SAFETY WARNINGS

Note: All steps with liquid Sigmacote should be done in a fume hood. Sigmacote contains hexane and should be disposed of properly in accordance with applicable regulations. Ashbya mycelia should be killed before disposal to the waste stream.

BEFORE START INSTRUCTIONS

Make Sigmacote coated test tubes (takes 24 hours).
Start a petri plate of Ashbya mycelium on AFM agar 5-7 days ahead of when you want to isolate spores.

Preparation of Sigmacote tubes

1 Add 250µl of Sigmacote to a glass screw cap tube.

2 Distribute the Sigmacote inside the tube by tipping and rotating the tube. Place in a roller drum for 1 hour (can be done at room temperature up to 37ºC).

3 Remove the excess Sigmacote by pouring off the liquid to a waste container. Let the open tubes air dry overnight in the fume hood.

4 Replace the screw caps loosely, then autoclave the tubes on a dry autoclave cycle.

5 The silicone is now covalently bonded to the glass and tubes are ready for use.

Spore isolation procedure

6 Start a petri plate of mycelium 5-7 days ahead of when you wish to isolate spores.The mycelium should be nearing the edge of the plate, but not be too old.

7 Add 10 mL sterile ddH_2O to a Sigmacote coated glass tube. Use the large end of a flat toothpick to scrape A gossypii mycelium of the agar plate and put it in the tube. Try not to get too much agar in with the mycelium, it will make pipetting difficult later in the protocol.

8 Shake and vortex the tube vigorously to break up the mycelium as much as possible (it will not break down completely but should be in many small pieces).

9 Incubate tube for 1 hour on a tube rotator or nutator at 37°C to allow spores to stick to the tube wall.

10 Discard the mycelia/water mix. Rinse the tube three times with 10mL with sterile ddH_2O to remove residual pieces of mycelium.

11 Add 10mL filter sterile 0.1% v/v Triton X-100 and shake and vortex vigorously to help dislodge the spores off the tube wall. Let sit for 10 minutes.

12 Invert tube once to gently resuspend spores and then transfer the liquid to a 15mL conical tube.

13 Centrifuge tube at 3000rpm for 4 minutes in a swinging bucket rotor. Spores will form a whitish pellet at the bottom of the tube. Carefully discard supernatant by inverting the tube.

14 Wash spores with 5mL filter sterile 0.03% v/v Triton X-100 and centrifuge at 3000rpm for 4 min.

15 Repeat wash.

16 Resuspend spores in 500µL filter sterile 0.03% v/v Triton X-100 + 500µL filter sterile 50% glycerol and transfer to screw cap tubes (100-200µl per tube depending on your usage). Label tubes with the strain, date, and “clean” to differentiate from dirty spore preps.

17 Store at -80°C (no need to flash freeze).