

Spore preparation using Sigmacote coated tubes

Spores are prepared taking advantage of their high hydrophobicity

Preparation of the tubes

- Add 250 μ l of Sigmacote (toxic!) to a glass test tube with screw cap
- Distribute it all over the tubes, remove the rest and let dry over night at 37 °C
- Autoclave the tubes
- The silicon is now covalently bound to the glass

Collecting the spores

- Add 15 ml sterile H₂O to the tube and add the mycelium of one plate.
Using a glass slide works best to scratch off the mycelium. Adding 1 ml of 100 % glycerol may improve the efficiency of washing the spores down, later.
- Shake vigorously to destroy the mycelium
- Let incubate 1 – 3 hours in a turning wheel to give the spores time to stick to the wall
- Discard the water and rinse the tube 3 x with pure water to remove residual pieces of mycelium
- Add 5 ml 0.1 % Triton and shake and vortex vigorously to wash the spores down from the glass wall.
- Transfer to a 15 ml Falcon tube
To maximize the yield, the test tube can be washed with another 5 ml of 0.1 % Triton and the solution added to the Falcon tube.
- Collect the spores by centrifugation
- Wash them 2 – 3 x with spore buffer (0.03 % Triton, and take them up in 500 – 1000 μ l of spore buffer.
The washing steps may be important to guarantee proper growth properties of the spores.
For time-lapse, make sure most of the Triton is removed or the spores will not germinate (e.g. dilute them with growth medium or let them grow in 50 ml AFM before adding them to the time lapse slide)!

Cleaning the tubes

- Add a standard cleaning agent and brush them using a small bottle cleaner,
- or let them wash by the cleaning personal
- Recoat them as described (incl. autoclaving!)

This protocol allows for the preparation of highly purified spore suspensions without enzymatic digestion. It is well suited for the isolation of spores from poorly sporulating strains.