

***A. gossypii* Actin staining (ALEXA FLUOR™ PHALLOIDIN)**

Incubation

- put 10 ml AFM into a 100 ml baffled flask
- add 100 – 200 μ l **fresh** spores (originating from 1 – 2 plates)
- add 20 μ l G418 (100 mg/ml stock)
- 10 μ l Amp (50 mg/ml stock) against contamination
- incubate 9 – 11 h at 30 °C, 150 rpm to obtain nice young mycelium, 8 h for unipolar germlings

1st Fixation

- put 1 ml Formaldehyde 37 % (**stored in the dark!**) + 10 μ l Triton 10 % into a 15 ml Falcon tube
- add 9 ml culture from flask
- mix carefully (don't vortex!)
- incubate 10 min in the dark

2nd Fixation

- collect the mycelium by centrifugation in Heraeus Minifuge: 1 min at 1000 rpm
- discard the supernatant
- resuspend the pellet in 900 μ l PBS and transfer it to 1.5 ml Eppis
- add 100 μ l Formaldehyde 37 %
- incubate 1 h in the dark, mix from time to time (don't vortex)

Washing out the Formaldehyde

- collect the mycelium in table top centrifuge: accelerate to 8000 rpm and let it stop right afterwards
- discard the supernatant
- resuspend the pellet in 1 ml PBS
- repeat

Stain

- resuspend the pellet in 100 μ l PBS
- add 10 μ l *Alexa Fluor™ Phalloidin* (6.6 μ M). The dye is **toxic!**
- mix carefully and incubate 1 h in the dark

Washing out the dye

- Wash 1 – 2 x with PBS (see step 4))

Preparing the slide

- add 1 drop of mounting medium on a slide
- add 4 μ l of the stained culture into the drop and mix on the slide using the yellow tip

- add the cover slip and leave it in the dark for 15 to 30 min to let it flatten.

Fluorescence Microscopy

Alexa FluorTM Phalloidin 488 is excited at 495 nm and emits at 518 nm. Use the FITC filter in combination with the XBO (Xenon) lamp to investigate your samples.

Note: Fluorescence will be lost, if the samples are exposed to short wavelight too long (photo bleaching).

Mounting medium

Dissolve 100mg p-phenylenediamine in 10ml PBS and bring to the volume of 100ml with glycerol. Mix thoroughly and store at -70°C.

To simultaneously observe nuclei, 50ng/ml Dapi can be added to the mounting medium