

Tubulin staining in *Ashbya*

- Fix a 50 ml overnight culture for 1 hour in 5.5 mls 37% formaldehyde, gently shaking at 30°. Transfer to 50 ml falcon tube and pellet by centrifuging at 3000 rpm for 15 minutes.
- Treat immunofluorescence slides with poly-lysine to make cells attach to surface. (10µl/well for 5 min, aspirate, let air dry, wash 1X with H₂O, aspirate and let air dry)
- Remove supernatant. Wash cells once in 50 mls 1X PBS in 50 ml falcon tube. Pellet by centrifugation. (If have very few cells, can also transfer to eppis and wash 2X in PBS there)
- Resuspend in 2 mls Solution A. Transfer 1ml of these cells to a new tube and add 150 µl 10 mg/ml zymolyase and 10µl BME and incubate with gentle rotation at 37° until see many phase dark hyphae (60-70% of cells). This may take 45-60 minutes. Put remainder of fixed, undigested cells at 4° for storage (good for up to one week).
- Digested cells are fragile, so take care when washing or they explode. Centrifuge at low speed in small bench top centrifuge. Wash cells 2X with 1 ml Solution A and resuspend in 500-1000 µl Solution A.
- Drop 20µl of cells onto a poly-lysine treated well of multi-well slide. Allow cells to settle for 15 minutes and then aspirate off supernatant. Let air dry completely.
- Wash cells 2 times with 1X PBS.
- To block cells, add 10µl 1X PBS+1mg/ml BSA(IgG free) and let sit for 30 minutes. Wash cells 2X with PBS+BSA.
- Add 10µl primary anti-Tubulin antibody at 1/50 dilution in PBS+BSA and place slide in humid chamber. Incubate 1-2 hours. (Rat anti-alpha tubulin YOL34 from Serotec)
- Wash 10X in 1X PBS+BSA
- Incubate 1 hour in secondary antibody+DAPI. Wash 10X in PBS + BSA.
- Mount slide in mounting medium and seal edges of coverslip with nail polish.

<u>Solution A</u>	<u>100mls</u>
0.1 M potassium phosphate buffer pH 7.5	10mls(1M)
1.2 M sorbitol	60mls(2M)
H ₂ O	30mls

Filter sterilize

Mouting medium

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