**Yeast Immunofluorescence**

Prepare poly-L-lysine coverslips in a 6-well dish. Add 1ml of poly-L-lysine (should be enough to cover the coverslip) and leave 30 min.

Pipette off excess (return to poly-L-lysine Falcon Tube) and wash with water. Wash with ethanol and air dry.

Start with 4ml culture OD ≈0.5. 4ml culture will be good for one coverslip for a considerable cell density.

Add 440µl 37% formaldehyde (from MERCK), incubate 15 min at room temperature.

Meanwhile make 10ml buffer B per staining.

Spin cells 2min at 4600rpm, pour liquid in formaldehyde waste.

Resuspend in 1ml buffer B, transfer to a 2ml tube.

Spin 2min at 5200 rpm.

Wash twice more with 1ml buffer B.

Resuspend cells in 100µl buffer B containing 1µl DTT 1M and 3µl high grade lyticase. Use cut tips.

Incubate 15 min at room temperature.

Pellet gently (1min 1000g) and pipette off supernatant. Wash with 1ml buffer B and repeat the centrifuge step.

Resuspend in 40µl buffer B.

Spot 40µl cells on poly-L-lysine coated coverslips and leave them for 20 min or more.

Wash with buffer B. Check cells in inverted microscope.

Add 2ml -20ºC methanol for 6 min. Use tweezers to dunk the coverslips in a small beaker with -20ºC acetone for 10 seconds and transfer to a new 6-well dish with PBS. Be quick to avoid over-incubation in methanol.

Wash 3x 2 min PBS in the wells. Check cells in inverted microscope.

Block with 1ml PBS + Triton X-100 0.3% + milk[[1]](#footnote-1) 5% during 30 min at room temperature.

Wash briefly 2x with PBS

Add primary antibody diluted in 50µl PBS + 1% BSA + 0.3% Triton X-100 to an empty slide. Use tweezers to remove each coverslip from the 6-well dish and place face-down on a spot of antibody solution. Close chamber and incubate overnight.

Wash in the wells 3x in PBS.

Add secondary antibody in the same solution, **in dark**. Incubate for 30 min at room temperature.

Wash in the wells 3x with PBS over an extended time, 30min to 1h should be enough to reduce background.

Dehydrate by washing each sample with:

* 70% ethanol for 3 minutes
* 90% ethanol for 3 minutes
* 100% ethanol for 3 minutes

Air dry the slides in paper towels.

Mount with 15 μl DAPI-vectashield.

Solutions

Buffer B: 0.1M potassium phosphate pH 7.5

 1M sorbitol

 100ml for 4 slides (18.2g sorbitol, 8ml K2HPO4, 2ml KH2PO4)

Lyticase stock: 15U/μl lyticase (Sigma >2000 U/mg L2524)

 10mM KPi pH7

 50% glycerol

 Store at -20°

 **Note calculate lyticase stock using U/mg solid**

1. You need to block yeast with milk – serum or BSA don’t work [↑](#footnote-ref-1)