Immunofluorescence of Aspergillus

Preparation of 16% formaldehyde solution.

Add 8 grams of paraformaldehyde to 40 ml of distilled water at 60°C. With constant stirring. add 5-10 drops of 1M NaOH until the paraformaldehyde dissolves. Some residual undissolved solids will remain. Bring the solution to 50 ml, then filter through a 0.45 μm filter to remove the solids. Prepare the buffered fixative solution relatively quickly, as neutral formaldehyde is more stable than the alkaline formaldehyde. An alternative is to purchase electron microscopy grade 16% formaldehyde from a commercial supplier.

Fixative:

1M PIPES-KOH pH 6.7	5 ml
0.25M EGTA pH 7 with KOH	10 ml
1M $MgSO_4$	0.5 ml
Dimethyl Sulfoxide (DMSO)	5 ml
16% formaldehyde solution	50 ml
Water	29.5 ml

Store at 4°C and use within 10 days.

Enzyme Solutiom:

Driselase	100 mg
Potassium Citrate, 100 mM, pH 5.8	920 μl
Leave on ice 15 minutes, then centrifuge at 2000 g to remove the starch carrier. To the supernatant, add:	
Novozyme 234	10 mg
0.25 M EGTA, pH 7	80 μl

Freeze immediately in 100-200 μl aliquots at –70°C.

1. Fix cells attached to cover slip for 45 minutes at their incubation temperature.
2. Wash in PEM (100mM PIPES pH6.7, 25mM EGTA, 5mM $MgSO_4$) 3 x for 5 minutes.
3.Drain the excess liquid from the coverslip.
4.Invert the cover slip onto a 200 μl portion of enzyme mix and egg white mixed 1:1. It is suggested that you place the digestions in the 25°C incubator so that there will be less variation between the different digestions. To determine th optimal digestion time for each lot of Novozyme 234, vary the incubation time from 30 to 90 minutes and remove a coverslip and process for immunofluerescence.
5. Wash the coverslips in PEM once for a 5 minutes and then 2 x for 10 minutes. The first wash in a large volume will rapidly stop the enzyme digestion.
6. Place the coverslips in PEM plus 0.1% NP-40 or Triton X-100 for 5 minutes.
7. Wash the coverslips 5 minutes in PEM, then 15 minutes in fresh PEM.
8. Place the coverslips sample side down on 200 μl of primary antibody diluted in PEM. Use the primary and secondary antibodies at the vendor's recommended concentration or at half that concentration. Allow the reaction to go one hour.
9. Wash 3 x 10 minutes in PEM .
10. Place coverslip on 200 μl drop of secondary antibody. Incubate for one hour at room temperature..
11. Was 3X 10 minutes in PEM.
12. If nuclear staining is desired, place the coverslip on a 200 μl drop of DAPI solution (15 ng/ml in water.). Incubate 10 minutes.
13. Dip the coverslip in water, drain briefly, then mount on 6 μl Citifluor AF1 or other antifade moounting medium.