Immunoflourescent assay

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Protist Research to Optimize Tools in Genetics (PROT-G) Julius Lukes

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 Preferably night before, make 4% paraformaldehyde (w/v) in PBS and incubate O/N at 37°C

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3 Spin down the cells 3000RPM for 2min and discard the supernatant in formaldehyde waste

4 Mix the pellet with 500ul of fixer in the hood and incubate the slides for 10min in humid dark chamber at room temperature

5 Remove excess of the paraformaldehyde by tilting the slide on the paper

6 Permeabilization with chilled methanol for 30min than 0.2% 1XPBS triton for 20min in dark

7 Slightly wash the cells with 1x PBS to remove detergent and make it air dry

8 Incubate the permeabilized cells with 5%milk prepared with 1X PBS+0.05% Tween 20 for 30min to 2h

9 Incubate the cells with primary antibody diluted in 5%milk (1X PBS+0.05% Tween 20) at 4°C

10 Wash the slides 3times with 1X PBS before adding secondary antibody diluted in 1% BSA (w/v) and incubate for 1h

11 Wash the slides 3 times in 1X PBS for 5min and tap away the moisture, to let it air dry

12 Drop on each sample 1 drop of Prolong Gold Antifade with DAPI(Invitrogen P36935)

13 Mount the slide with coverslip and sail with nail polish

14 Let it dry in the humid chamber for 10 min and can store in dark at 4°C until observing under the microscope.

15 Wash the slides 3times with 1X PBS before adding secondary antibody diluted in 1% BSA (w/v) and incubate for 1h. After incubation washes the slides 3X times with 1X PBS. Make it air dry.

16 Add one drop of Prolong Gold Antifade with DAPI(Invitrogen P36935) Mount the slide with the coverslip and sail with nail polish.

17 Let it dry in humid chamber for 10min and so on.

18 Store it in dark at 4°C until use