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Glyoxal fixation of mammalian cells for immunofluorescence

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ABSTRACT

This is our standard protocol for glyoxal fixation of mammalian cells grown on glass cover slips for immunofluorescence microscopy.

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MATERIALS TEXT

Standard materials to make the required buffers:
ddH2O
PBS
Ethanol
Glyoxal, 40%
Glacial acetic acid
BSA
Ammonium chloride

Mountant containing DAPI

All solutions can be made up to one day in advance and stored in the fridge.

Glyoxal solution
- 2.835 ml ddH2O
- 0.789 ml ethanol (absolute)
- 0.313 ml glyoxal (40% stock solution)
- 0.03 ml glacial acetic acid (100%)

Adjust the pH to 5-6 with 1M NaOH using pH paper

Quenching solution
- 0.535 mg NH4Cl

Adjust to 100ml with PBS

Blocking solution (PBS with 2.5% BSA and 0.1% Triton X-100)
- 2.5g BSA
- 1 ml Triton X-100 (10% stock solution)

Adjust to 100 ml with PBS

Antibody buffer (PBS with 1% BSA and 0.1% Triton X-100)
- 1g BSA
- 1 ml Triton X-100 (10% stock solution)

Adjust to 100 ml with PBS

Mountant with DAPI

Fix the cells 5m

1 Wash the cells with warm DMEM without FCS. 5m

2 Add 300µl **glyoxal solution** per well (12-well plate) and incubate on ice for 30 minutes **On ice** **00:30:00**, then at room temperature for 20 minutes **Room temperature** **00:20:00**. 40m

3 Wash 2 times with PBS 10m

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4 Add 300µl **quenching solution** for 20 minutes **Room temperature** **00:20:00** 20m

5 Wash once with PBS 5m

At this stage, fixed cells can be stored in the fridge for at least a week.

Staining the cells 2h 35m

6 Add 500µl **blocking solution** and incubate at room temperature for 30 minutes **Room temperature** **00:30:00** 30m

In meantime, prepare the primary antibody. The dilution is dependent on the antibody, but can range from undiluted for non-purified hybridoma supernatants to 1:1000 for affinity purified antibodies. The antibodies are diluted in **antibody buffer**.

7 There are two ways to incubate the antibodies: 45m
1. Prepare enough so that the slide can be covered in the well with the antibody solution, ±250-300µl.
2. To save antibody, prepare only 50µl and pipette this onto parafilm. Place the coverslip on top of this drop *with the cells facing the drop* and incubate in a humid chamber.

Incubate at least 45 minutes at room temperature or alternatively overnight at 4ºC **Room temperature** **00:45:00**

8 Place the coverslips back in the 12-well plate with *the cells facing upwards*. 15m

Wash 3 times with PBS.

9 Dilute the secondary antibody in **antibody buffer**, generally in a range of 1:1000-1:2000 for most antibodies. Add 250-300µl per well and incubate for at least 45 minutes at room temperature or alternatively overnight at 4ºC **Room temperature** **00:45:00** . 45m

IMPORTANT: this incubation step should be in the dark, as the conjugated secondary antibodies are light-sensitive.

10 Wash 2 times in PBS, followed by 2 times in ddH2O. 15m

11 Place a drop of mountant containing DAPI on a microscope slide and place the coverslip on this drop with the *cells facing the drop*. 5m

IMPORTANT: warm up the mountant to 37ºC to avoid unequal DAPI-staining. Tip the coverslip sideways on a tissue to remove excess water before mounting.

12 Dry the slides overnight in a dark place at room temperature. Store at 4ºC afterwards until imaging.

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