Immunfluorescence Protocol

Solutions and reagents

Lysis buffers

10 x PBS:

- 80 g NaCl
- 2 g KCl
- 14 g Na_2HPO_4
- 2.4 g $KH_2PO_4$15O mM NaCl

1. Resolve in 800 ml ddH_2O.
2. Adjust pH to 7.4 using HCl.
3. Fill it up to 1 l.
4. Autoclave it.
5. Store at RT.
6. Dilute 1:10 before use.

PBS-T:

- 1 x PBS + 0.1% Tween20

Permeabilization solution:

- 0.3% Triton X-100 in PBS

Blocking solution:

- 1.5 g BSA
- 50 ml PBS-T

1. Resolve BSA in 50 ml PBS-T (dissolves well at 37°C waterbath).
2. Optional: add 2.5% normal serum matching host species of secondary antibody.

Hoechst33258 (4 mg/ml Stock):

1. Dilute 1:4,000 in PBS.

Kaisers Glyceringelatine

Procedure

Fixation and optional permeabilization of cell membrane

1. Fixation of cells or tissue:
    a. 10 min at -20°C with Acetone or
    b. 15 min at RT with 3% PFA.
2. Wash for 5 min with 1x PBS pH 7.4 at RT in a Coplin Jar.
3. Allow the sample to dry for a few minutes.
4. Mark the region around the sample with a PAP Pen and let it dry.
5. If an intracellular staining is performed permeabilize the membrane with 0.3% Triton X-100/PBS for 10 min at RT.
6. Wash for 5 min with 1x PBS pH 7.4 at RT in a Coplin Jar.

Staining

1. Block the sample with 3% BSA in PBS and incubate in the dark in a humidity chamber for 20 min at RT.
2. Wash the slide once for 5 min with 1x PBS-T pH 7.4 RT in a Coplin Jar.
3. Put the slide in the dark in a humidity chamber.
4. Add a suitable dilution of primary antibody (diluted in blocking solution) to each sample.
5. Incubate at 37°C for 30 min, followed by incubation at RT for 30 min (alternative incubate at 4°C over night).
6. In general double labeling can be performed by applying two appropriate primary antibodies (from different species) simultaneously.
7. Drain off antibody solution and rinse for a short time with distilled water.
8. Wash twice for 5 min with 1x PBS p H 7.4 at RT in a Coplin Jar.
9. Briefly rinse in distilled water.
10. Put slide back in the dark in a humidity chamber.
11. Add suitable dilution of secondary antibody (diluted in blocking solution) to each sample.
12. Incubate at 37°C for 30 min, followed by incubation at RT for 30 min.
13. Drain off the antibody solution and rinse for a short time with distilled water.
14. Caution: perform all the following steps in the dark!!! (fluorochromes are light sensitive, place Coplin Jar in a drawer to protect from light).
15. Wash twice for 5 min with 1x PBS pH 7.4 at RT in a Coplin Jar.
16. Briefly rinse in distilled water.
17. Add Hoechst33258 (4 mg/mL Stock) 1:4,000 dilution in PBS.
18. Incubate for 2-3 min in a dark humidity chamber.
19. Quick wash twice with 1x PBS-T pH 7.4 at RT in a Coplin Jar.
20. Quick wash with MilliQ-Wasser.
21. Warm Kaiser's Glycerol Gelatin in a water bath.
22. Wipe the slide dry from below.
23. Add a few drops on a cover slip and place the slide over, press slightly to remove air bubbles.
24. Press the slides between tissue paper with weight over (Eppendorf stand and bottle of water) for at least 2 hours.
25. Slides are ready for microscopy.