Immunostaining of cultured cells using SyliteD1.1

Materials (not included):

- Phosphate Buffered Saline (PBS) pH 7.4
- Paraformaldehyde (PFA):
  4% PFA in PBS pH 7.4, freshly prepared [1]
- Quenching solution (QS):
  0.1 M Glycine in PBS pH 7.4
  or: 0.1 M NH_4Cl in PBS pH 7.4
- Blocking & Permeabilization buffer (BPB) [2]:
  10% Normal Goat Serum (NGS) + 0.1% Triton X-100 in PBS
  or: 2% Bovine Serum Albumin (BSA) + 0.1% Triton X-100 in PBS.
- SyliteD1.1 Dilution Buffer (SDB) [2]:
  3% NGS + 0.1% Triton X-100 in PBS
  or: 1% BSA + 0.05% Triton X-100 in PBS
- High-salt PBS:
  PBS supplemented with 0.5 M NaCl

Procedure

The example below is based on a 12-well plate.
Please adapt the protocol to your experimental conditions.

1. Wash cells gently using PBS (e.g. 1 ml of PBS per well).
2. Add 1 ml of 4% PFA per well and incubate at room temperature (30 min, RT).
3. Remove PFA and dispose according your laboratory rules.
4. Briefly rinse with 1 ml QS per well.
5. Add 1 ml of fresh QS per well and shake gently on an orbital shaker (10 min, RT).
6. Remove QS.
7. Briefly rinse with 1 ml of PBS per well.
8. Add 1 ml of BPB per well and shake gently (15 min, RT).

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9. During this time prepare the SyliteD1.1 working solution. Make sure to prepare sufficient volume for all reactions (e.g., 5 ml for a full 12 well plate).
    a. Resuspend lyophilized SyliteD1.1 in 50% Glycerol to have a stock solution.
    b. Dilute the SyliteD1.1 reagent in SDB to the best working dilution [(3)].
10. Remove BPB solution from wells.
11. Add 400 µl per well of the SyliteD1.1 working solution. Incubate for 60 minutes with gentle shaking at RT and protected from light.
12. Remove the SyliteD1.1 working solution from well.
13. Rinse once with 1 ml of PBS per well.
14. Wash with 1 ml of PBS per well and shake the plate gently for 5 min at RT and protected from light.
15. Repeat the previous step 2 times.
16. Optional step
    a. Wash once for 5 min with high-salt PBS.
    b. Briefly rinse with PBS.
17. SyliteD1.1 is a short peptide that does not tolerate well many mounting media. The best signal to-noise ratio is obtained if imaged when samples are freshly prepared and on PBS.
18. Optional step
    a. If mounting is required, please post-fix SyliteD1.1 with a new round of 4% PFA.
    b. Repeat steps 2 – 7
    c. Rinse your sample in distilled water to remove salts and proceed to mount your sample.

Remarks

[(1)] SyliteD1.1 is not compatible with methanol or glutaraldehyde fixation.

[(2)] We recommend using blocking and SyliteD1.1 dilution buffers prepared with Normal Goat Serum (NGS), if BSA is use, we suggest to filter buffer (before adding Triton X-100) through a 0.2 or 0.45 µm syringe filter.

[(3)] To obtain optimal results for different samples (primary neuronal cultures, brain slices, etc.), the dilution factor might need to be adjusted. The recommended dilution specified in the data sheet is thus only a starting point for further optimizations.

For further information concerning this protocol please contact us at

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Bench protocol

1. Fix cells with 4% PFA for 30 min at RT.
   SyliteD1.1 is not compatible with methanol or glutaraldehyde fixation.

2. Quench with PBS supplemented with 0.1 M glycine or 0.1 M NH_4Cl in PBS for 10 min at RT.

3. Rinse once with PBS.

4. Permeabilize and block for 15 min with 10% Natural Goat Serum (NGS) and 0.1% Triton X-100 in PBS.

5. Dilute SyliteD1.1 with 3% NGS and 0.1% Triton X-100 in PBS.
   To obtain optimal results for your sample the dilution of SyliteD1.1 might need to be adjusted.
   The recommended dilution is thus only a starting point for optimization

6. Incubate fixed cells with the diluted SyliteD1.1 for 60 min at RT.

7. Wash 3 times for 5 min each with 1 ml of PBS

8. Optional: Wash once with high-salt PBS (PBS + 500 mM NaCl) followed by PBS.

9. Image your SyliteD1.1 staining freshly and directly on PBS (or similar buffer)

8. Optional: Post-Fix SyliteD1.1 to allow the use of mounting media. Repeat Steps 1-3.

9. Shortly dip coverslip in water before mounting.
   We recommend using Mowiol as a mounting medium.

Reference

Khayenko, V. et al. **A Versatile Synthetic Affinity Probe Reveals Inhibitory Synapse Ultrastructure and Brain Connectivity**. *Angewandte Chemie Int Ed* (2022) doi:10.1002/anie.202202078.

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