General protocol Immunofluorescence

Protocol may need local adjustments

Step 1 Rinse cells attached to cover slips twice with PBS, removing liquid by gentle aspiration in this and subsequent steps.

Step 2 Fixation & permeabilization:

Option I:

Fix cells with 4% formaldehyde in PBS for 6 min at room temperature, then rinse briefly twice with PBS**.

Permeabilize fixed cells with 0.2% Triton X-100 in PBS for 6 min.

Option II:

Fix/permeabilize cells in -20°C methanol for 6 minutes**.

[**Note: At this stage, the procedure may be interrupted for long term storage. Coverslips immersed in PBS and stored at 4°C are good for several weeks.]

Step 3 Wash cells briefly 3 times with PBS, then 2 times with PBS containing 5% BSA (blocking reagent).

Step 4 Dilute primary antibody in PBS/5% BSA. Working quickly, aspirate area surrounding coverslip to dryness, then gently add 100 µl of diluted primary antibody to the coverslip, so that solution remains restricted to coverslip by surface tension. Incubate for 1 hour at room temperature in a moist environment to prevent drying.

Step 5 Wash cells 3 times with PBS, then 2 times with PBS/5% BSA.

Step 6 Dilute fluorochrome-coupled secondary antibody in PBS/5% BSA and apply as in step 5. Incubate 1 hr. at room temperature.

Step 7 Wash cells 3 times with PBS, then mount coverslips to slides using antifade mounting medium.

Nordic Biosite AB
Propellervägen 4A | 183 62 TÄBY, SWEDEN
+46 (0)8 5444 3340 | info@nordicbiosite.com

Considerations

- Adherent cells may be grown directly on coverslips or chambered slides; suspension cells may be adhered to coverslips via poly-L-lysine treatment.

- Care should be taken to use the highest quality primary and secondary antibodies in order to avoid non-specific labeling. Ideally the specificity of primary antibodies is confirmed via immunoblotting of cell extracts. A control immunofluorescence sample omitting the use of primary antibody will reveal the extent of non-specific signal generated by the secondary antibody.
- In case of high background, the use of less primary and/or secondary antibodies as well as increased or alternative blocking reagent can be considered. 10% serum in PBS is another useful blocking agent - use only serum that will not cross-react with secondary antibody.

- If the assay involves localization of a protein expressed from a heterologous promoter, then the researcher should keep in mind that overexpression of the protein may produce mislocalization and hence broader staining than expected from endogenous expression.

- Several approaches can be considered in cases of an unacceptably low signal. The immunofluorescence protocol itself may be altered: use increased amounts of primary antibody, extend the incubation of primary antibody to overnight at 4°C, or use a different fixation/permeabilization regimen (gluteraldehyde, acetone, others).