ICC: Staining Protocol - Formaldehyde Fixation

This protocol is suitable for the immunocytochemical analysis of formaldehyde (FA) fixed cells. Scientists use self-made FA fixation solutions produced by dissolving paraformaldehyd (PFA) in phosphate buffered saline (PBS), or they apply ready-to-use FA fixation solutions containing different amounts of methanol for stabilization. In our standard ICC protocol, we apply a ready-to-use FA solution with a low amount of methanol. The optimal fixation time may vary depending on cell-type, cellular compartment or antibody. Too short or too long fixation times may lead to bad cell structure integrity or masking of antigens. In our standard protocol, we apply 15 min 4% FA fixation to obtain good results. However, some antibodies need shorter / milder FA fixation or even methanol or acetone fixation, because the epitope is prone to be masked by FA crosslinking (please check the remarks sections for deviating fixation protocols).

Important: Some proteins have special requirements for good detection. Please refer to the **remarks** sections for ICC on the respective data sheet.

Materials and reagents

- **PBS:** Phosphate buffered saline, pH 7.4
- **Fixation buffer**: 4% FA (alternatively 4% PFA) in PBS, pH 7.4, 4% sucrose
- **Blocking buffer:** 10% normal serum, 0.1% Triton X-100 in PBS (normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Incubation buffer:** 5% normal serum, 0.1% Triton X-100 in PBS (Triton may be omitted; normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Mounting medium**
- **Optional:** DAPI nuclear stain

Procedure

1. Wash cells briefly with PBS.
2. Fix cells with **fixation buffer** for 15-20 min at RT.
3. Wash three times with PBS for 10 min.
4. Incubate for 30 min with **blocking buffer**.
5. Incubate in **incubation buffer** containing the primary antibody (for appropriate dilution, refer to the data sheet) for 2 h at RT.
6. Wash three times with **PBS** for 10 min.
7. Incubate in **incubation buffer** containing the secondary antibody for 1 h at RT (optimal dilution must be determined experimentally). **Optional:** Add DAPI to the secondary antibody solution. *Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye.* **Important Note:** This step can be omitted when fluorophore conjugated primary antibodies are used. In **multiplex** staining, make sure to use secondary antibodies cross-adsorbed against the host species of the other primary antibody used in your experiment. Ideally, all secondary antibodies should come from the same host species. If not, make sure that they have been cross-adsorbed against IgGs of the host-species of the other secondary antibody as well. This avoids cross-reaction between the secondary antibodies.
8. Wash three times with **PBS** for 10 min.
9. Mount coverslips and observe under a microscope.

Note: *The SYSY standard protocol generates good results in the SYSY labs and may be used as a reference. However, to achieve the highest specific signal and lowest non-specific background signal, the best antibody concentration, incubation temperature, and incubation time must be determined individually.*