ICC: Staining Protocol - Methanol Fixation

Methanol fixation works by denaturing and precipitating proteins, and as such it is a quick method. For most antibodies/proteins, it takes only 5 minutes. This procedure sometimes leads to an unmasking of epitopes.

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

- **Methanol fix:** Ice cold methanol (*stored at -20°C)*
- **PBS**: Phosphate buffered saline, pH 7.4
- **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, (normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Mounting medium**
- **Optional:** DAPI nuclear stain

Procedure

1. Carefully cover cells with **methanol fix** and incubate for 5 min at -20°C.
2. Wash three times with PBS for 10 min.
3. Incubate with **blocking buffer** for 30 min.
4. Incubate in **incubation buffer** containing the primary antibody (for appropriate dilution, refer to the data sheet) for 2 h at RT.
5. Wash three times with **PBS** for 10 min.
6. Incubate in **incubation buffer** containing the secondary antibody (optimal dilution must be determined experimentally) for 1 h at RT. **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 antibodies as well. This avoids cross-reaction between the secondary antibodies.
7. Wash three times with **PBS** for 10 min.
8. 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.*