

## Reference Protocol for Anti-Ki67 (HS-398 102) Immunocytochemistry of Methanol Fixed Cells

### 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:** 3% normal serum, 0.1% Triton X-100 in PBS, (normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Secondary antibody:** anti-rat secondary antibody conjugated to fluorescent dye
- **Optional:** DAPI (4 mg/ml)

### Procedure:

1. Cover cells carefully with **methanol fix** and incubate for 5 min at -20°C.
2. Wash three times for 10 min in **PBS**.
3. Incubate for 30 min with **blocking buffer**.
4. Apply the primary antibody in **incubation solution** at a 1:1000 dilution and incubate for 2 h at RT.
5. Wash three times for 10 min in **PBS**.
6. Apply the secondary antibody in **incubation solution** diluted to the manufacturer's recommended concentration and incubate for 1 h at RT. **Optional:** Add DAPI at a 1:10,000 dilution to the secondary antibody solution.

*Note: Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye. In Multiplex stainings 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.*

7. Wash three times for 10 min in **PBS**.
8. Mount coverslips and observe under a microscope.

*Note: The SYSY standard protocol generates good staining 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 for each antibody must be individually determined. Please also refer to our general protocols.*