Immunofluorescence Cell Staining

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1. Take out the coverslip and drain it onto Kimwipes.

2. Put the coverslip in 6-well plate having 3% paraformaldehyde (PFA) and let stand for 15 min.

3. Wash cells 3X with PBS for 10 min.

4. Replace fixing solution with 0.2% Triton X-100/1% normal goat serum (NGS) in PBS and let stand for 5 min on ice.

5. (Optional) When staining nucleic acid, after 3X 10-min washing with PBS, add dyes, let stand for 5-10 min and wash 2x with PBS, 10 min each

6. Wash cells 3X with 1% NGS in PBS, 10 min each.

7. Transfer coverslip to a piece of parafilm set on a flat surface. Add 100 μl of primary antibody diluted in 5% NGS in PBS. Incubate at RT for 1 hr.

8. Remove the antibody solution from a coverslip, and wash 3X with 200 μl of 1% NGS in PBS, 10 min each.

9. Add 100 μl of secondary antibody diluted in 5% NGS in PBS to coverslips, and incubate at RT for 1 hr.

10. Wash 3X with 200 μl of PBS, 10 min each.

11. Mount the coverslip on the slide with Vectashield.
    - 12 mm round coverslip: 3 μl of Vectashield or 4 μl of Prolong
    - 18 mm rectangle coverslip: 8 μl of Vectashield or 10 μl of Prolong

* Approximately 2 ml of 5% NGS in PBS is required for the staining of each coverslip.