Protocol for the Immunostaining of Biotin-Streptavidin System

1. Fix the cells with 2-4% paraformaldehyde containing 1mM Na3VO4 at RT for 15-30min.
2. 3x 5min/each wash in PBS containing 1mM Na3VO4.
3. Permeabilize with 0.1-0.5% TX-100 in PBS containing 1mM Na3VO4 for 15-30 min, at RT
4. Apply 1-2 drops of the streptavidin (molecular probes, E-21390), incubate for 15-30min at RT
5. Rinse thoroughly with PBS containing 1mM Na3VO4, 1x wash 5min
6. Apply 1-2 drops of biotin, incubate for 15-30min, at RT
7. Rinse thoroughly with PBS containing 1mM Na3VO4, 1x wash 5min
8. Incubate with 3% goat normal serum or 1% BSA for 20 min, at RT
9. Wash 1x PBS, 5 min/each, at RT
10. Incubate primary antibody (1:100, 10ug/ml) in (optional, 0.1%TX-100, 1% goat normal serum), at RT for 2 hr or 4oC O/N, in PBS containing 1mM Na3VO4.
11. Wash 3X PBS, 5min/each, at RT
12. Incubate biotinylated anti-mouse (from goat) (1:200 or 1:100) in (optional, 0.1%TX-100) PBS, at RT for 1 hr
13. 3x PBS wash, 5 min/each, at RT
14. Incubate FITC-streptavidin (1:200) in PBS, at RT for 30 min
15. 3x PBS wash, 5min/each, at RT
16. Optional: double staining, continue routine immunostaining if primary abs are from Rabbit.
    - Caution! If primary Abs are fromGoat, do Goat Staining first and keep 1mM Na3VO4 in all the solution used.
17. Mount