



ICL002.0P - Virus Antigen Immunostaining for Influenza (5/01/2009)

Protocol:

1. Fix the cells with 3.3% formalin (10% solution diluted in PBS). Incubate at RT for 1hr or at 4°C over night.
2. Discard the supernatant and add 0.1% Triton X-100 in PBS.
3. Incubate at room temperature for 30 min. Discard the supernatant.
4. Add 1% BSA in PBS. Incubate at room temperature for 30 min or at 4°C over night. Discard the supernatant.
5. Add 100 µl primary antibody in 1% BSA-PBS. (For influenza use anti-NP monoclonal antibody 3/1, 1:2000 dilution.)
6. Rock at room temperature for 1hr.
7. Discard the supernatant.
8. Wash cells with 2ml PBS three times over 10 min. Discard the supernatant.
9. Add 100 µl biotinylated anti-mouse IgG (H+L) (Vector Laboratories) in 1% BSA-PBS (1:200 dilution).
10. Rock at room temperature for 1hr. Discard the supernatant.
11. Wash cells with 2 ml PBS three times over 10 min. Discard the supernatant.
12. During the wash step, mix 10ul each of solution A and B from Vectastain ABC kit (Vector Laboratories) in 1ml of 1% BSA-PBS.)
13. Add staining mixture to the cells.
14. Rock at room temperature for 30 min. Discard the supernatant.
15. Wash cells with 2 ml PBS three times over 10 min. Discard the supernatant.
16. During the wash step, prepare DAB solution. Add 1 tablet of 3,3'-Diaminobenzidine tetrahydrochloride (DAB) to 10 ml PBS and vortex. (Note: DAB tablet does not dissolve completely).
17. Add 10 ul of 30% hydrogen peroxide to 10 ml of DAB solution, vortex, and add to cells.
18. Incubate at room temperature until the brown color develops (usually several minutes)
19. Wash cells with distilled water.
20. Air-dry. Capture images with a light microscope

Media and Reagents:

1. Formalin buffered 10%, Fisher Cat# SF100-4



2. Triton X-100, Sigma, Cat# T9284
3. BSA, Sigma, Cat# A4503
4. Biotinylated anti-mouse IgG (H+L), Vector Laboratories, Cat# BA-9200
5. Vectastain ABC kit, Vector Laboratories, Cat# PK-4000
6. Diaminobenzidine tetrahydrochloride (DAB, Sigma Cat# D5905)
7. 30% hydrogen peroxide, Fisher, Cat#H325-100

