Immunohistochemical staining of Osteopontin and Osteocalcin on Cultured Cells

(Liang Chen, 4/18/2009)

1) Rinse cells with PBS.

2) Fix cells with Acetone or 10% Formalin. 10min (Acetone is the optimal choice, 10% Formalin is reasonable)

3) Wash cells with PBS, 1min×3 times.

4) Dry cells in Air for 10min.

5) Block with 5% BSA, 20min, then briefly rinse with PBS.

6) Block with Avidin-Blocking Solution, 15min. Then, briefly rinse with PBS.

7) Block with Biotin-Blocking Solution, 15min. NO WASH!!!

8) Incubate with 1st Antibody; concentration of the antibody refers to 1:100, 1:200, 1:300. (The concentration is depended on the quality of your 1st antibody) 1 hour, Room temperature.

9) Wash with PBS, 1min×3 times.

10) 10. Incubate with 2nd Antibody, 1:600 Bioinylated α-mouse IgG in PBS containing 2% mouse serum. 30min, Room temperature.

11) Wash with PBS, 1min×3 times.

12) Incubated with 1:200 HRP-Streptavidin in PBS. 30 min, Room temperature.

13) Washed with PBS, 1min×3 time.

14) Developed immuno-staining with DAB, 7min. (Observe the sample for each 3-5min) (Fresh dissolved one of each tablet in 5ml Di-Water, then, add 2.5ml PBS to 7.5ml total volume before used.)

15) Washed with Tap Water→Di-Water once briefly.

16) Counter-stained with Mayer's Hemotoxylin Solution, 1-2min.

17) Washed with Tap water→NH_4OH wash→Tap water.

18) Observe and take photos under microscope in 1-2 days. Samples should be kept in 4°C