PROTOCOL FOR IMMUNOSTAINING OF BETA-CATENIN IN CULTURED CELL LINES

Hesheng Ou 9/20/00, Edited by JYP 8/13/02

Note: This protocol is for cultured cells in 48-well plate.

1. Cell cultured on 48-well plate.
2. Remove the media.
3. Fix the cells with methanol: 250 μl/well, -20^{0}C, 15 minutes.
4. Wash × 2 with PBS (add PBS, incubate 5 minutes, remove PBS = wash).
5. Add 1% NP-40, 250 μl/well, RT, 10 min.
6. Wash × 2 with PBS.
7. Add goat serum 200 μl/well, 30-60 minutes, RT.
8. Remove the serum.
9. Add whole goat serum containing primary antibody; 1: 250, 250 μl/well, RT, 1 hour
10. Wash 2 × with PBS
11. Add secondary antibody; 1: 2000, 250 μl/well, RT, 1 hour
12. Wash 2 × with PBS (note: for DAB staining, see below)
13. Add streptavidin-Alexa; 1: 500, 200 μl/well, RT, 30 min
14. Wash 2 × with PBS
15. Add PBS 400 μl/well
16. Proceeding under microscopy.

For immunocytochemistry (DAB staining):

1. Proceed with the same steps of 1-12 above.
2. The secondary antibody is 1:100~1000.
3. Add DAB mixture, 250ul/well.
4. Monitor the color develop (dark brown) under the microscopy.

For ABC staining:

1. After secondary antibody (Biotin-labeled), add “Linker”, 4 drops into the well, incubate for 20min at RT.
2. Wash 2 X with PBS.
3. Add “Label”, 4 drops, for 20min incubation at RT.
4. Wash 2 X with PBS.
5. Add the substrate solution to develop the color.
6. Wash with PBS to stop the color development.