IMMUNOHISTOCHEMICAL STAINNING ON CULTURED CELLS

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This protocol is for cultured cells in 48-well plates.

1. Plate cells in 48-well plates;
2. Remove the media.
3. Fix the cells with methanol: 250 μl/well at -20^{0}C for 15 min.
4. Wash with PBS × 2 (add PBS, incubate 5 min, remove PBS = wash);
5. Add 1% NP-40 (prepared in PBS), 250ul/well at room temperature for 10 min;
6. Wash with PBS × 2;
7. Add goat serum 200ul/well for 30-60 min at RT;
8. Remove the serum;
9. Add whole goat serum containing primary antibody 250ul/well @ RT for 60min. Always accompany with a negative control without primary antibody (**Note**: different titer may have to be tested, e.g., usually in the range of 1:100 to 1:500);
10. Wash with PBS x2;
11. Add biotin-labeled secondary antibody (usually at 1:2000 to 1:10000), 250ul/well at RT for 20-30min;
12. Wash with PBS x2
13. Use one of the following method to develop the staining:

For immunofluorescent staining with Alexa (Molecular Probe)

14. Add streptavidin-Alexa (usually at 1:500 to 1:5000), 200ul/well at RT for 30min;
15. Wash with PBS x2-3;
16. Add PBS 400ul/well;
17. Wrap with aluminum foil and record staining under fluorescence microscopy.

For immunohistochemical DAB staining (BioGenex Kit)

1. Proceed the above Steps 1-10;
2. Add “Muitilinker” (i.e., biotin-labeled secondary antibody), 2-4 drops to each well. Incubate for 20min at RT;
3. Wash 2X with PBS;
4. Add “Label” (i.e., Streptavidin-conjugated with HRP), 2-4 drops per well, and incubation at RT for 20min;
5. Wash with PBS x2;
6. Add the DAB Substrate Solution (PIERCE) 300ul/well to develop the color;
7. Wash with PBS to stop the color development (few seconds to 20min, depending on signals). Keep staining with PBS and store at 4°C.