BT312 Lab_Immunocytochemistry Protocol

Reagent preparation

1. **1X Phosphate Buffer Saline (PBS):**

   Dissolve the contents in 800 ml of MiliQ water, Adjust the pH to 7.4 and then make up to 1 litre, followed by autoclaving.

Reagent	Amount	Final Concentration
NaCl	8g	137 mM
KCl	0.2g	2.7 mM
Na_2HPO_4	1.44g	10 mM
KH_2PO_4	0.24g	1.8 mM

2. **4% Paraformaldehyde (PFA):**

   4g of paraformaldehyde in 80 mL of 1X PBS.

   - Take the mixture to fumehood, put on a magnetic stirrer, heat upto 60 °C and stir continuously.
   - Add 1N NaOH dropwise, till the powder completely dissolves (approx. 200 µL for 100mL)
   - Then, let the solution cool, adjust the pH to 6.9 using dilute HCl.
   - Make up the solution to 100 mL, filter it, and then aliquot and store in -80 °C

3. **Triton-X:**

   0.1% Triton™ X-100 is prepared in 1X PBS

4. **Blocking sol:**

   0.5% bovine serum albumin, 0.15% glycine dissolved in 1X PBS

5. **Hoechst dye:**

   1:10,000 dilution in PBS

6. **Primary Antibody:**

   Vimentin; Dilution 1:1000 in blocking solution

7. **Secondary Antibody:**

   Alexa Fluor 488, Anti-Rabbit; Dilution 1:2000 in blocking solution

Protocol:

1. Cells in the 24 well plate are washed twice with phosphate buffer saline (1X PBS, 500 µL) and fixed with 4% paraformaldehyde (250 µL) for 15 mins.
2. Wash the cells with 500 µL of 1X PBS twice.
3. Fixed cells are then permeabilized by treating with 0.1% Triton™ X-100 in PBS for 15 minutes (250 µL).
4. Wash the cells with 500 µL of 1X PBS twice.
5. After permeabilization, cells are blocked with a blocking solution (250 µL) for an hour at room temperature.
6. The primary antibody (200 µL) is added and incubated overnight at 4 °C in a moist chamber.
7. Cells are washed twice with 1X PBS and then incubated with the corresponding secondary antibody (200 µL) for an hour in a moist chamber at room temperature.
8. After incubation, cells were washed two times with 1X PBS and then stained with Hoechst 33342 (1:10,000; Invitrogen) for 5 minutes.
9. Excess staining is removed by washing with 1X PBS thrice and then visualized under an inverted fluorescent microscope.