

Immunofluorescence Protocol (example for GRP78)

Purpose:

The purpose of the IF is to make a fluorescing image of a tissue sample and to learn about the location of a certain protein in the tissue. If there are proteins uniquely expressed by a certain type of cell, the IF can show the location of that type of cell.

Protocol:

1. Xylene wash (**15 mins**)
 - a. Wash 3 times with xylene (5 mins each)
2. Graded ethanol washes (**35 mins**)
 - a. Wash 2 times in 100% ethanol (10 mins each)
 - b. Wash in 95% ethanol (5 mins)
 - c. Wash in 70% ethanol (5 mins)
 - d. Wash in 50% ethanol (5 mins)
3. PBS Wash (**10 mins**)
 - a. Wash 2 times with PBS (5 mins each)
4. Antigen Retrieval/Unmasking Antigen (**30-45 mins**)
 - a. Make sodium citrate solution
 - i. Use a magnetic stirrer
 - ii. 4.41g sodium citrate
 - iii. 1.5 L ddH₂O
 - iv. Add 1N HCl until the pH reaches 6.0
 - b. Allow sodium citrate solution to warm up in pressure cooker
 - c. Using the black slide holder, submerge slides in solution
 - d. Set pressure cooker to high for 5 minutes
 - e. Allow solution to cool
 - f. Wash 2 times with PBS (3 mins each)
5. Blocking (**60 mins**)
 - a. Make Incubation Buffer (IB)
 - i. 750 ul PBS
 - ii. 200 ul 15% BSA
 - iii. 20 ul donkey serum
 - iv. 3 ul Tween-20
 - b. Make Blocking Buffer (BB)
 - i. 475 ul IB
 - ii. 50 ul 15% BSA
 - iii. 12.5 ul donkey serum
 - c. Outline samples with ImmEdge pen
 - d. Incubate with BB at room temperature (60 mins)
 - i. shake off excess blocking buffer (do not wash)
6. Primary antibody (**overnight**)

- a. Dilute primary antibody with IB as required (1:40 dilution for grp78)
 - b. Incubate with primary antibody overnight in 4 degree fridge
 - c. On the negative control, incubate with only IB
7. PBS wash (**30 mins**)
 - a. Wash 3 times in PBS (10 mins each)
8. Secondary antibody (**60 mins or overnight**)
 - a. Dilute secondary antibody with IB (1:1000 dilution)
 - b. Incubate with secondary antibody in 4 degree fridge overnight, or at room temperature for 60 mins
9. DAPI stain (**45 mins**)
 - a. Wash in PBS (5 mins)
 - b. Dilute DAPI with PBS (1:1000 dilution)
 - c. Apply DAPI (5 mins)
 - d. Wash 3 times in PBS (10 mins each)
10. Mount slides with coverslips, tapping any air bubbles out