Please cite the J.B. Lawrence Lab or publications for use of this protocol. Thanks!

Antibody Staining + RNA Hybridization

Usually we do the antibody reaction first, but some antibodies only work if they come last (same protocol but reversed).

Primary AB

1. Rinse coverslips in 1xPBS 10 min

2. Make appropriate dilution of antibody in 1xPBS/1% BSA and add 1unit/ul of RNAsin. Place a 50-80µl drop onto a glass plate lined with parafilm

3. Place coverslip, cell side down, on top of drop

4. Cover with a 2nd piece of parafilm, seal the sides like an envelope to prevent evaporation and incubate 1 hour at 37°C.

5. Wash coverslips at room temp:
   1xPBS – 10min on shaker
   1xPBS + 0.1% Triton – 10min on shaker
   1xPBS – 10min on shaker

Secondary AB

6. Make a appropriate dilution of conjugated (Fitc, Texas Red, Alexa 488 or 594) antibody in 1XPBS/ 1% BSA add 1unit/ul of RNAsin.

7. Place drops of secondary antibody onto a parafilm lined glass plate

8. Place coverslip on top of drop, cells down

9. Cover with a 2nd piece of parafilm, wrap entire plate with tin foil, (to keep reaction in the dark) and incubate 1 hour at 37°C.

10. Wash coverslips at room temp :
    1xPBS – 10min on shaker

1xPBS + 0.1% Triton – 10min on shaker
1xPBS – 10min on shaker

11. Fix coverslips in 4% Paraformaldehyde for 10 min, room temp, in dark

12. Rinse twice in 1xPBS

RNA hybridization

13. Follow Lawrence Lab standard RNA hybridization protocol from this website.

14. Use a detector with a different fluorochrome than the one used with the antibody.

DAPI DNA stain

15. Incubate in DAPI stain, 30sec-1 min, in dark

16. Rinse twice with 1xPBS

17. Mount coverslips onto slides using Vectashield (Vector Labs) mounting media and seal edges with fingernail polish.

18. Slides are stored in a slide folder at -20°c