Immuno-FISH (Adapted from Titia de Lange)

Blocking Solution
10% BGS in 1X PBS

Antibody diluting Solution
-1% BSA/0.1% tritonX-100 in 1X PBS
(I have also just used blocking solution to dilute the antibody).

Hybridizing Solution (from Q-FISH)

Stock	For 150µl total
Tris 1M, pH 7.4	1.5
Buffer $MgCl_2$	12.84
Deionized formamide (Sigma, F9037-100ML)	105
Probe 25µg/ml (tel probe)	3
10% Blocking reagent	7.5
Distilled water	20.16

Make fresh

Washing Solution (from Q-FISH)

Stock	For 40 ml
Formamide	28
Tris 1M pH7.2	0.4
BSA 10% (in water)	0.4
Water	11.2

Make fresh

Buffer $MgCl_2$ (from Q-FISH)

Stock	For 100 ml
1M $MgCl_2$	2.5
0.1M citric acid	9.0
1M Na_2HPO_4	8.2
Water	80.3

Make 10-15 ml aliquots and store at -20ºC

Blocking reagent (from Q-FISH)

1. Prepare Maleic Acid buffer : 100 mM maleic acid, 150 mM NaCl, adjusted to pH 7.5 with NaOH.
2. Dissolve 10g of blocking reagent (Boehringer catalog # 1093 657) in 100 ml Maleic acid buffer pH 7.5. Difficult to dissolve, use heat (heating block or microwave).
3. Make 5-10 ml aliquots and store at -20ºC.

IF-FISH

1. I start out with poly-l-lysine coated microscope cover glasses in tissue culture dishes (immerse the cover glass in poly-L-lysine for ten minutes, then aspirate to remove the poly-l-lysine).

    - For MEFs I use either 22 x 22 cover glass (in p-100 plates), or 9 x 9 (in p-60 plates).
    - This can be altered to use twelve welled dishes with the 9x9 coverslips.

2. Plate cells so they are subconfluent (70-80%) the following day.

For the following steps I leave the cover glass in the tissue culture plate, with minimal agitation during washes. Cells will detach relatively easily prior to fixation.

3. Wash 3x 5min in 1X PBS at room temp ☐ ☐ ☐

4. Fix in 3.7% paraformaldehyde/0.2% Tx-100/1X PBS: 10 minutes at room temp ☐

5. Wash in 1X PBS: 3x 5min ☐ ☐ ☐

6. Incubate (in a humidity chamber) for 1hr at 37^{o}C in blocking solution ☐

7. Wash 3x in 1X PBS, 5 minutes ☐ ☐ ☐

   Important: carefully aspirate all the PBS from around the coverslips.

8. Incubate (in a humidity chamber) for1hr in primary antibody diluted 1:400, at 37^{o}C ☐

    - Put the diluted antibody directly on top of the coverslips (ensure that it covers it). It usually does not spill over to the sides if you have aspirated all the PBS from the area immediately surrounding the coverslip.

    - 22x22 coverslips require up to 150-200 ul, while 9x9 require 40-50ul.

9. Wash in 1X PBS: 3x 5min ☐ ☐ ☐

10. Incubate 1hr in secondary antibody (Alexa-fluor) diluted 1:400 in blocking solution at 37^{o}C ☐

11. Wash in 1X PBS: 3x 5min ☐ ☐ ☐

12. Fix in 3.7% paraformaldehyde/1X PBS for 10 min at room temp ☐

13. Wash extensively in 1X PBS: 3 x 10min ☐ ☐ ☐

14. dehydrate in 70%, 90%, 100% ethanol ☐ ☐ ☐

15. Air dry coverslips for 10-20 min ☐

16. For each coverglass, put a drop (8 ul) of hybridizing solution onto a microscope slide (you can do 2-3 cover glass per slide). Add the cover glass to the hybridizing solution, facedown on the slide. ☐

17. Denature at 80^{o}C for 6 minutes ☐

18. Incubate in the dark, 4hrs at room temp, or 4 degrees overnight. ☐

19. Using washing solution, gently rinse coverslips from slides, so they fall facing upwards, into the tissue culture dishes. ☐

20. Wash the coverslips 2x 15 minutes with washing solution ☐ ☐

21. Wash 2x 5min in 1X PBS ☐ ☐

22. Air dry slides ☐

23. Counterstain with DAPI and mount ☐

Notes – when air drying the coverslips, they can adhere to the plate and become really difficult to remove. I find that it's easier to first put them at a 45 degree angle against the side of the tissue culture dish while they are drying.