**IF-FISH**

**Required Solutions**

**Blocking solution**
(in PBS, store at -20°C in 10 ml aliquots)
1 mg/ml BSA
3% goat serum
0.1% Triton X100
1 mM EDTA pH 8.0

**Blocking reagent** (Roche 11096176001)
Stock is 10%, dissolved in maleic acid buffer:
100 mM malie acid
150 mM NaCl
Adjust pH to 7.5 (20°C) with NaOH. Store at 4°C

**Hybridizing solution**
Make fresh. Final concentrations in dH₂O.
70% formamide (from deionized stock)
0.5% blocking reagent (from 10% stock)
10 mM Tris-HCl pH 7.2
FITC-TelC (1:500-1000) or Tam-TelG (1:3000)

**PNA probes** (Custom from BioSynthesis)
Tamra-TelG: Tam-OO-TTAGGGTTAGGGTTAGGG 3’
Stock concentration: 53 µM in dH₂O
Store at 4°C in the dark.
For FISH analysis, use 1:3000-5000 dilution dH₂O

FITC-TelC: FITC-OO-CCCTAACCTAACCCTAA 3’
Stock concentration: 111 µM in dH₂O
Store at 4°C in the dark.
For FISH analysis, use 1:500-1000 dilution dH₂O

**Washing solution**
70% formamide
10 mM Tris-HCl pH 7.2

**3000x DAPI**
Dissolve 0.5 mg/ml 4’,6-diamino-2-phenylindole (Sigma D-9542) in H₂O.
Stable for at least one year at 4°C.

**Embedding medium**
Dissolve 20 mg p-phenylene diamine (Sigma P-6001) in 2 ml 10xPBS by vortexing.
Immediately add 18 ml glycerol, mix carefully without creating air bubbles. Do not vortex.
Store in 1 ml aliquots at -70°C.
* or use ProLong Gold Antifade Reagent (Invitrogen #P36934)
IF-FISH Protocol

1. Grow cells on cover slips to sub-confluence. Aspirate medium and wash in PBS.
2. Fix the cells for 10 min at rt in 2% paraformaldehyde or for 10 min at -20ºC in 100% cold methanol.
3. Wash 2 x 5 min in PBS.
4. Wash 5 min in PBS (optional). Cells can be stored at 4ºC at this point, add 1000x stock NaN₃ from 10% stock in H₂O. Use caution, NaN₃ is poisonous.
5. Incubate cover slips for 30 min in blocking solution.
6. Incubate 1 hour in primary antibody diluted in 50-100 µl blocking solution.
7. Wash 3 x 5 minutes in PBS.
8. Incubate 30 min in secondary antibody* diluted in 50-100 µl blocking solution.
9. Wash 3x 5 minutes in PBS.
10. Fix the cells on the cover slips for 5 min at rt in 2% paraformaldehyde.
11. Wash 2x 5 minutes each in PBS.
12. Dehydrate the cells in ethanol, consecutively 70%, 95%, 100% EtOH, 5 min each. Aspirate the ethanol completely and let the cover slips dry for a couple of minutes.
13. Place a drop of hybridizing solution (60-80 ml) on each cover slip.
14. Denature with hybridizing solution for 3-10 min at 70-80ºC by placing slides on heat block.
15. Incubate in the dark for 2 hours at rt or over night at 4ºC.
16. Wash 2 x 15 min in washing solution.
17. Wash 3 x 5 min in PBS; add DAPI to the second wash.
18. Air dry at rt for 10 minutes.
19. Embed and seal.

* Notes:
- If using TelC telomeric probe, match with RRX-conjugated secondary antibody.
- If using TelG telomeric probe, match with Alexa 488-conjugated secondary antibody.
- If you wish to co-stain two proteins and telomeres, combine TelC with red Cy3 and Far-red Cy5 (weaker staining).