Interphase FISH on Cultured Cells

This is a baseline protocol. Some steps may need to be optimized by the researcher.

This protocol is optimized for mammalian cells.

All solutions are made with molecular grade DNase/RNase free water unless otherwise specified.
All steps are at room temperature (RT) unless otherwise specified.
All slide washes are done in coplin jars/slide dishes with gentle agitation/rocking.

For reconstitution of shipped myTags *in situ* hybridization probes see:
myTags Product Reconstitution Protocol

Pretreatment of Superfrost Plus slides (Fisher Scientific cat#1 2-550-15)

1. Preclean microscope slides (even if noted as "pre-cleaned) for 2 minutes in 100% ethanol.
2. Air dry slides, cover to prevent dust from collecting on slides.
3. Incubate slides in 0.01% (v/v) poly-L-Lysine solution for 5 minutes in a plastic coplin jar/slide dish.
4. Air dry slides as in step 2.

Cell preparation on slide

1. Prepare cell suspension of 0.5-2 X 10^6 cells/ml in growth media.
2. Add 100 µl of cell suspension per slide roughly in the center.
3. In humid environment to prevent drying allow the cells to adhere for 1-3 hours at the growth parameters of the cell line (37°C, 5% CO_2 for mammalian cells).
4. Rinse briefly with 1X PBS.
5. Fix slides for 5-15 minutes in 4% (v/v) paraformaldehyde in 1X PBS in coplin jar/slide dish.
6. Rinse briefly with 1X PBS.
7. Rinse for 5 minutes in 2X SSCT.
8. Rinse for 5 minutes in 2X SSCT + 50% (v/v) formamide.
9. Transfer to fresh 2X SSCT + 50% (v/v) formamide in a coplin jar/slide dish for storage at 4°C.
10. Slides are best used within 1-2 weeks of generation.

Interphase FISH on Prepared Slides:

1. Prewarm appropriate vol of 2x SSCT + 50% (v/v) formamide to 92°C and 60°C.
2. Remove coplin jar/slide dish containing slides from 4°C and allow them to warm to RT.
3. Rinse slides in prewarmed 92°C 2x SSCT + 50% (v/v) formamide for 2.5 minutes.
4. Rinse slides in prewarmed 60°C 2x SSCT + 50% (v/v) formamide for 2.5 minutes.
5. Remove the slides from the coplin jar/slide dish and allow them to cool to RT. Cover to prevent dust from collecting on slides.
6. Add 25 µl of hybridization solution with 20 pmol* myTags probe to a 22x22 #1.5 coverslip.
7. Invert the slide onto the coverslip ensuring the cells are covered.
8. Seal the coverslip with rubber cement or appropriate sealant (Cytobond; ScieGene cat# 202-00-1) and let cure for 5 minutes.
9. Incubate the slide in humid environment at 92° for 2.5 minutes.
10. Transfer slides to humid environment at 37°C or 42°C and hybridize overnight.
11. Prewarm appropriate vol of 2X SSCT to 60°C.
12. Following hybridization remove coverslip carefully and rinse the slides in prewarmed 2X SSCT for 15 minutes.
13. Rinse the slides in 2X SSCT at RT for 10 minutes.
14. Rinse the slides in 0.2X SSC at RT for 10 minutes.
15. Proceed to nuclear staining. Recommend Hoechst 33342 (Invitrogen Cat #H3570).
    - Dilute stock (10 mg/ml) 1:2000 in PBS for final concentration of 5 µg/ml
    - Incubate slide 30 sec and rinse in PBS.
16. Coverslip with anti-fade media.
    - Recommend Prolong Diamond (Invitrogen Cat #P36961).
    - Cure at RT overnight before imaging.
17. Store slide at -20°C for long term storage.

REAGENTS

Fixative

4% Paraformaldehyde in 1X PBS – make fresh

- 4 ml of 10X PBS
- 26 ml molecular grade H_2O
- 10 ml 16% paraformaldehyde

2X SSCT

2X SSC with 0.1% Tween-20

- 50 ml 20X SSC
- 500 µl Tween 20
- Bring to volume of 500 ml with molecular grade DNase/RNase free H_2O
- Store at 4°C, 1 month shelf life

0.2X SSCT

0.2X SSC with 0.1% Tween-20

- 5 ml 20X SSC
- 500 µl Tween 20
- Bring to volume of 500 ml with molecular grade DNase/RNase free H_2O.

- Store at 4°C, 1 month shelf life

4X Hybridization Buffer
40% Dextran Sulfate, 8X SSC, 0.8% Tween-20

- 8 ml 20X SSC
- 8.5 ml molecular grade DNase/RNase free H_2O.
- 8 g Dextran Sulfate powder
    - Mix overnight by inversion on rotary shaker then add;
- 160 µl Tween-20
- Bring to volume of 20 ml and mix overnight as before.

Hybridization Mix
2X SSCT, 50% Formamide, 10% Dextran Sulfate, 10 µg RNase A

- 50 µl Deionized Formamide
- 25 µl 4X Hybridization Buffer
- 4 µl 10 µg/µl RNase A (in molecular grade DNase/RNase free H_2O)
- Enough myTags probe/s in molecular grade DNase/RNase free H_2O at 20 pmol* to bring volume to 100 µl.

Reaction mix volume can be scaled down as long as myTags probe amount is at appropriate pmol

*Appropriate pmol amount of myTags probe may need to be optimized. Recommended starting amount is 20 pmol/rxn.

Adapted from:
Beliveau BJ, Boettiger AN, Nir G, Bintu B, Yin P, Zhuang X, Wu CT. In Situ Super-Resolution Imaging of Genomic DNA with OligoSTORM and OligoDNA-PAINT. Methods Mol Biol. 2017;1663:231-252. doi: 10.1007/978-1-4939-7265-4_19. PMID: 28924672; PMCID: PMC5919218.

Wu Lab-Harvard Medical School-Department of Genetics-Protocols