

Interphase FISH protocol

1. Remove coplin jar(s) containing slides from 4 °C allow them to warm to room temperature
2. Incubate slides in 2X SSCT + 50% (v/v) formamide for 2.5' at 92°C in a pre-warmed coplin jar
3. Transfer slides to a coplin jar containing 2X SSCT + 50% formamide at 60°C and incubate for 20'
4. Remove the slides from the 60°C coplin jar and allow them to cool to room temperature
5. Add 25 µl of a hybridization cocktail composed of 2X SSCT, 50% formamide, 10% (w/v) dextran sulfate, 10 µg RNase A, and 10-20 pmole of Oligopaints probe to a 22x22 #1.5 coverslip
6. Invert slides onto the cocktail-containing coverslips such that the area containing the cells is covered. Seal with rubber cement
7. Allow the rubber cement to air-dry for 5' at room temperature
8. Denature for 2.5' at 92°C by placing slides top of a water-immersed heat block inside a water bath
9. Transfer slides to a humidified chamber and hybridize overnight at 37°C or 42°C
10. The next day, remove the coverslip carefully and wash slides in a pre-warmed coplin jar containing 2X SSCT at 60°C for 15'
11. Transfer slides to a coplin jar containing 2X SSCT at room temperature and incubate for 10'
12. Transfer slides to a coplin jar containing 0.2X SSC at room temperature and incubate for 10'
13. Remove slides from the coplin jar and gently tap them dry on paper towels – take care to just tap the thin edge of the slide and to never allow the surface containing the cells to directly contact the paper towels
14. Add 15 µl of mounting media such as SlowFade Gold + DAPI to a 22x30 #1.5 coverslip
15. Invert slides onto mounting media-containing coverslips such that the area containing the cells is centered beneath the coverslip

16. Seal each slide using nail polish

17. Allow at least 30' for the nail polish to dry before imaging the slides to ensure no nail polish gets on the microscope objectives

18. In our hands, slides remain quite stable (e.g. for several months) when stored at 4°C and protected from light

2X SSCT - 0.3 M NaCl, 0.03 M NaCitrate, 0.1% Tween-20

0.2X SSC - 0.03 M NaCl, 0.003 M NaCitrate