Hybridization Conditions for Cytological Specimens

These are a suggested set of conditions to start with and could be adjusted on a per probe basis.

Cell Type:

Cell lines, peripheral blood or bone marrow specimens, both cultured and processed directly.

Sample Type:

Suggested for use with cells treated with hypotonic solution and fixed in methanol/acetic acid.

Slides:

If slides are made the day of hybridization, they have to be aged: baked at 90˚C for 10 minutes, and/or pre-treated in 2X SSC (either at 73˚C for 2 minutes or at 37˚C for 30 minutes). If slides have aged overnight at room temperature, no baking or 2X SSC pretreatment is necessary.

STEP	Fast Working Vysis Hybridization Buffer	LSI/WCP Vysis Hybridization Buffer
Hybridization Buffer	Mix Fast Working Buffer by pipetting up and down few times	Vortex, spin down
Probe Mix Preparation (the same amount of probes used per assay regardless of final volume)	• 12 µL hyb buffer • X µL probe(s) • (3-X) µL H_2O 1 assay: total volume **15 µL** **Mix by pipetting up and down**	• 7 µL hyb buffer • X µL probe(s) • (3-X) µL H_2O 1 assay: total volume **10 µL** **Mix by vortexing or pipetting**
Specimen Slide Dehydration-1	70% (v/v) ethanol for approximately 1 minute	
Specimen Slide Dehydration-2	85% (v/v) ethanol for approximately 1 minute	
Specimen Slide Dehydration-3	100% ethanol for a minimum of 1 minute (keep the specimen slides in 100% ethanol until ready to dry all slides and apply the probe mixture)	
Air Dry	Air dry	
Probe Application	Apply probe mix to target area, coverslip (22x22 mm), seal with rubber cement	
Codenaturation/Hybridization	On ThermoBrite (insert water saturated humidity strips prior to start of the assay)	
Automated Co-Denaturation	80˚C for 2 minutes	73˚C for 2 minutes
Hybridization	37˚C for **1 – 18 hours**	37˚C **overnight (12 - 24 hours)**
Wash 1 Buffer	0.4X SSC/0.3% NP 40	
Wash 1 Conditions	73˚C ± 1˚C for 2 minutes ± 30 seconds	

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STEP	Fast Working Vysis Hybridization Buffer	LSI/WCP Vysis Hybridization Buffer
Wash 2 Buffer	2X SSC/0.1% NP 40	
Wash 2 Conditions	Ambient for 5 – 60 seconds	
Air Dry	Air dry	
Counterstain	Apply 10 µL DAPI II and coverslip (22x22 mm)	
Hybridized Slides Storage	-20˚C protected from light	

NOTES: Applies to FFPE Assay Conditions

1. Fast Working Buffer is more viscous than LSI/WCP Buffer. Vortexing is not sufficient to mixprobes with the buffer.
2. ThermoBrite has to be cleaned between hybridizations. Take out slide-locating bar and remove rubber cement residues. Clean ThermoBrite heating surface with 70% alcohol prior to start of the assay to remove any particles that can interfere with the contact between heating surface and glass slides.
3. If processing less than 12 slides at once, fill empty slots on ThermoBrite with blank slides throughout incubation.
4. If washing only a few hybridized slides, blank room temperature slides can be added to the wash to help maintain temperature.