



# HYBRIDIZATION CONDITIONS FOR CYTOLOGICAL SPECIMENS

**Abbott**

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<br>(the same amount of probes used per assay regardless of final volume) | <ul style="list-style-type: none"> <li>• 12 µL hyb buffer</li> <li>• X µL probe(s)</li> <li>• (3-X) µL H<sub>2</sub>O</li> </ul> 1 assay: total volume 15 µL<br><b>Mix by pipetting up and down</b> | <ul style="list-style-type: none"> <li>• 7 µL hyb buffer</li> <li>• X µL probe(s)</li> <li>• (3-X) µL H<sub>2</sub>O</li> </ul> 1 assay: total volume 10 µL<br><b>Mix by vortexing or pipetting</b> |
| 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<br>(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<br>(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 mix probes 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.