Universal Pretreatment and Hybridization Conditions for FFPE Specimens

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

Sample Type:

FFPE tissues and cell lines. Any sample type that is embedded that requires digestion.

STEP	Fast Working Vysis Hybridization Buffer	LSI/WCP Vysis Hybridization Buffer
Baking	60°C for 2 - 24 hours (ThermoBrite or oven)	
Deparaffinnization	Hemo-De or Xylene Ambient 3 times 5 minutes each step	
Dehydration	100% Ethanol Ambient 2 times 1 minute each step	
Air Dry	Air dry	
Pretreatment	Pretreatment SSC Buffer 80°C ± 2°C for 25 minutes ± 15 minutes	
Rinse	Purified water Ambient for 3 minutes	
Pretreatment	50 mL Protease Buffer + 75 mg Protease IV (1 vial) Dissolve for 1 hour at 37°C 37°C ± 1°C for 20 minutes ± 10 minutes	
Rinse	Purified water Ambient for 3 minutes	
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	
Air Dry	Air dry	
Hybridization Buffer	Mix Fast Working Buffer by pipetting up and down few times	Vortex, spin down

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STEP	Fast Working Vysis	LSI/WCP Vysis
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**
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	73 ºC for 5 minutes	
Hybridization	37ºC for 2 – 3 hours	37ºC overnight (14 - 24 hours)
Post-Hybridization Wash Buffer	2X SSC/0.3% Tween 20	
Wash 1 Conditions	Ambient for approximately 5 minutes allow coverslips to float off; gently remove remaining cover slips	
Post-Hybridization Wash Buffer	2X SSC/0.3% Tween 20	
Wash 2 Conditions	73°C ± 1ºC for 3 minutes ± 1 minute	
Air Dry	Air dry	
Counterstain	Apply 10 µL DAPI I and coverslip (22x22 mm)	
Hybridized Slides Storage	-20°C protected from light	