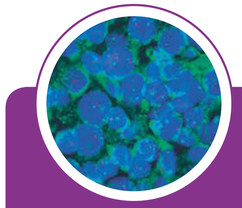
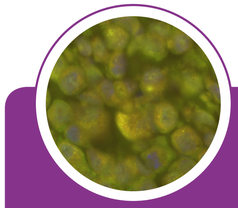
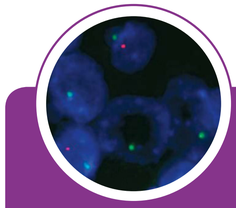
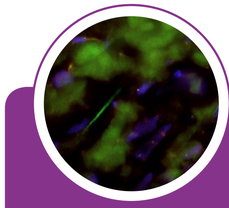
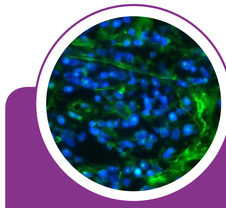
FISH 'n' Tips

What's wrong with my FFPE FISH?

A Sysmex Group Company

	MICROTOME SECTIONING	SLIDE MOUNTING	EXCESS PARAFFIN	INSUFFICIENT PRETREATMENT	OVER DIGESTION	UNDER DIGESTION	OVER DENATURATION	UNDER DENATURATION
								
PROBLEM								
IDENTIFIER	• FFPE sections tear very easily and become difficult to manipulate	• Paraffin wax begins to melt • Sections difficult to mount on the slide • Tissue does not adhere	• Excess paraffin will preferentially bind to FITC • Insufficient paraffin clearing affects the probe's ability to penetrate tissue	• High level of auto-fluorescence and background • Weak probe signals	• Auto-fluorescence • Doughnut or 'ghost' nuclei • Absence of DAPI staining in some areas • Destroyed tissue morphology • Loss of target gene signal in some cells	• A cloudy haze across the cells • Inconsistent DAPI staining • Reduced probe signal strength	• Tissue fragmentation • Degraded probe • High background	• Absent or very weak probe signals
SOLUTION	✓ Place tissue block on ice for 20 mins before sectioning to enable smooth cutting ✓ Regularly replace your cutting blade ✓ Longer, continuous ribbons are easier to manipulate	✓ Maintain water temperature of 40°C- higher temperatures melt the paraffin wax ✓ Use scalpels/ tweezers to manipulate the sections ✓ Ensure good adhesion using positively charged slides	✓ Ensure all paraffin is cleared with extra washes over a longer time period	✓ Store both solution and enzyme at 2-8°C ✓ Maintain temperature of water bath at 98-100°C for at least 15 mins ✓ Replace pre-treatment solution after each batch (max of 20 slides per wash) ✓ Use ceramic jars instead of glass to maintain the temperature ✓ Use plastic balls on the surface of the water bath to reduce evaporation	✓ Decrease the enzyme digestion time ✓ After digestion, stain with DAPI and check under microscope ✓ The number of over-digested cells should be <15% of the total	✓ Increase the enzyme digestion time ✓ Perform the step using a 37°C hotplate ✓ Ensure that the enzyme is stored correctly	✓ Calibrate hotplate/hybridiser to ensure that the correct denaturation temperature is achieved ✓ 75°C for 5 mins is recommended; however increasing the denaturation temperature up to 85°C may improve signal strength in difficult specimens	

Suggested Digestion Times:*

- Breast: 10-40 mins
- Lung: 15-20 mins
- Ovary: 10-20 mins
- Kidney: 20-25 mins
- Colon: 20 mins
- Brain: 15-18 mins

The optimal enzyme digestion time will depend on the age of the block, tissue composition and quality of fixation. Our technical support team are able to offer further help and guidance on optimising digestion times for your tissue.

HOTPLATE OR HYBRIDISER?

	HOTPLATE	HYBRIDISER
PROS	+ Immediate co-denaturation + Better temperature regulation	+ Easy-to-use, less manual input + High throughput
CONS	− Not ideal for high throughput processing	− Temperature inconsistencies across positions − Loss of humidity can lead to signal drop out; ensure that the humidity strips are pre-soaked in a 37°C water bath prior to hybridisation

For more troubleshooting tips, visit our FISH 'n' Tips page:
www.cytocell.com/support
contact@ogt.com

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*These timings are based on independent validation carried out by Cytocell on a representative set of tissues using the Cytocell Tissue Pretreatment Kit (LPS100) and should be used for guidance purposes only.

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