



F.A.Q., Tips and Troubleshooting

When viewing the results of a FISH essay, ensure that the microscope is properly aligned and functioning optimally. The following table lists some less than optimal results that may be encountered using our products. Probable causes and suggestions to improve assay performance are included.

Q: How can I storage Rapid-ISH or Smart-ISH products?

A: The optimal storage temperature range between RT and -20°C; if it is possible it is recommended to store at +4°C in the original vial

Q: Do I have to change probe firmly to use Rapid-ISH or Smart-ISH products?

A: No, all the products are compatible with the most common probes already used in the daily lab practice.

Q: Do I have to change my pre-treatment reagents and protocols to use Rapid-ISH or Smart-ISH products?

A: You can maintain your already used protocol and reagents both for Smart-ISH Solve, both for Rapid-ISH Integra.

For a better efficiency with Rapid-ISH Integra you can refer to the protocols reported in the Rapid-ISH datasheet or on www.oncology-and-cytogenetic-products.com/eng/tutorial.html.

Q: Which are the most appropriate denaturation time and temperature?

A: The usage of Rapid-ISH and Smart-ISH does not affect the probes denaturation parameters; refer to probes datasheet.

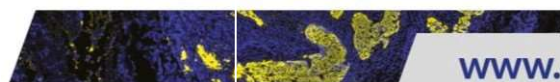
Q: Which are the most appropriate hybridization time and temperature?

A: The usage of **Smart-ISH Solve** does not affect probes hybridization parameters; refer to probes datasheet.

A2: The usage of **Rapid-ISH Integra** products does not affect the hybridization temperature (refer to probes datasheet), and allow to **reduce the hybridization time to 40 minutes**.

LIMITATIONS OF THE PROCEDURE

For the procedure limitations refer to probes datasheet.



Problem	Probable Cause	Possible Solution	
No signal or weak signals	Inappropriate filter set used to view slides	Use recommended filters.	
	Inappropriate hybridization time	Verify hybridization time	
	Inappropriate post-hybridization wash temperature	Verify temperature	
	Air bubbles trapped under coverslip prevented probe access	Apply coverslip by first touching the surface of the probe mixture.	
	Inadequate tissue digestion		Verify temperature of the digestion solution
			Verify time of the digestion step.
	Section over fixed (cell boundaries will be distinct)	Prolonged tissue fixation times may lead to progressive degradation of signal intensity and may require longer digestion times	
	Too low probe volume used vs sample dimension	Repeat the test using a little bit more probe volume	
Probes not well preserved	Change the probe vials		
Variation of signal intensity across tissue section	Probe unevenly distributed on slide due to air bubbles under coverslip	Repeat assay on next adjacent section of same tissue block and make sure no air bubbles are trapped under coverslip	
		Apply coverslip by first touching the surface of the probe mixture.	
Tissue loss or tissue morphology degraded	Tissue section under-fixed (poor DAPI staining)	Verify tissue digestion time	
	DNA loss (poor DAPI staining)	Verify fixation conditions	
		Verify Rapid-ISH Hybridization time	
	Inappropriate slides	Use positively-charged slides	
Over pretreatment	Verify time and temperature		
Problem	Probable Cause	Possible Solution	
Tissue loss or tissue morphology degraded	Tissue section was torn when removing coverslip after hybridization	Allow additional time for coverslip to soak off in wash buffer	
	Improper slide baking	Verify temperature	
	Over denaturation	Verify denaturation time	

