F.A.Q., Tips and Troubleshooting

When viewing the results of a FISH assay, 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: Have I to change probe firm 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: Have I 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 is 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 is 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.**

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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

LIMITATIONS OF THE PROCEDURE

For the procedure limitations refer to probes datasheet.