

SUGGESTED PROTOCOLS FOR FFPE SAMPLES

FOR THE MAXIMUM PERFORMANCE CHANGE YOUR REAGENTS BEFORE THE PROTOCOL STARTS

TISSUE DEWAXING: (Not necessary for Rapid-ISH Integra Recovery protocol)

- Place the slides in a dry oven at 65 °C for 30 minutes
- Place in a dry oven at 65 °C a coplin jar with 50 ml of Xylene
- Pre reheated a coplin jar with 100ml of SSC2X in the water bath at 77 °C
- Pre reheated a coplin jar with 100ml of SSC2X in the water bath at 47 °C
- Pre heat in the water bath a coplin jar with 100ml of SSC2X / 1.5% NP40 at 75 °C
- On the hybridization plate set the fixed temperature at 75 °C
- Incubate the slides on the plate at 75 °C for 5 minutes
- Immerse the slides in Xylene in oven at 65 °C for 30 minutes
- Proceed with 3 sequential washings of the slides with 50 ml of xylene in a coplin at RT for 3 minutes/each
- Dry the slides at RT for 5 minutes
- Dehydrate the slides in 2 sequential steps in coplin with 50 ml of 100% ethanol for 5 minutes/each
- Dry the slides at RT for 5 minutes

RAPID-ISH INTEGRA, RAPID-ISH INTEGRA PLUS & SMART-ISH TISSUE TREATMENT:

- Incubate the slides in a coplin with 2X SSC at 77 °C for a time to be determined between 7 and 18 minutes in relation to the characteristics of the sample
- Dissolve 630ul of Proteinase K in the coplin with SSC at 47 °C
- Incubate the slides in the coplin at 47 °C for a time to be determined between 7 and 18 minutes in relation to the characteristics of the sample
- Then wash the slides in a quick dip into a coplin with 50 ml of SSC2X
- Dehydrate the slides in 3 sequential steps in a coplin with 50 ml of Ethanol 70% -85% -100% for 1 minute/each
- Dry the slides at RT for 5 minutes

RAPID-ISH INTEGRA RECOVERY TISSUE TREATMENT:

- Wash the slides in a quick dip into a coplin with 50 ml of SSC2X
- Dehydrate the slides in 3 sequential steps in a coplin with 50 ml of Ethanol 70% -85% -100% for 1 minute/each
- Incubate the slides in a coplin with 2X SSC at 77 °C for a time to be determined between 30 seconds and 2 minutes in relation to the characteristics of the sample.
- Dissolve 630ul of Proteinase K in the coplin with SSC at 47 °C
- Incubate the slides in the coplin at 47 °C for a time to be determined between 30 seconds and 2 minutes in relation to the characteristics of the sample
- Then wash the slides in a quick dip into a coplin with 50 ml of SSC2X
- Dehydrate the slides in 3 sequential steps in a coplin with 50 ml of Ethanol 70% -85% -100% for 1 minute/each
- Dry the slides at RT for 5 minutes

RAPID-ISH HYBRIDIZATION STEPS:

- On each slide affix 1 to 5 ul of probe and 5ul of the appropriate Rapid-ISH Buffer
- Cover the area with a cover slip and seal with rubber cement
- Set on the hybridization plate a protocol which provides: Denaturation, temperature and time according to the probes specifications; Hybridization, temperature according to the probes specifications,
- **TIME: 40 MINUTES**

SMART-ISH HYBRIDIZATION STEPS:

- On each slide affix 1 to 5 ul of probes and 5ul of Smart-ISH Buffer
- Cover the area with a cover slip and seal with rubber cement
- Set on the hybridization plate a protocol which provides: Denaturation, temperature and time according to the probes specifications; Hybridization, temperature according to the probes specifications,
- **TIME: O/N**

POST HYBRIDIZATION STEPS:

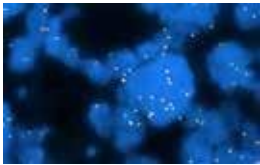
- Remove the coverslip and quickly wash slides in a Coplin with 50 ml of SSC2X at RT
- Dip the slides in the coplin with SSC2X / 1.5% NP40 at 75 °C for 3 minutes
- quickly wash slides in a coplin with 50 ml of SSC2X at RT
- Dehydrate the slides in 3 sequential steps in a coplin with 50 ml of Ethanol 70% -85% -100% for 1 minute/each
- Dry the slides at RT for 5 minutes
- Affix 5-10 ul of DAPI on each slide, cover with a coverslip
- Ready for the observation under the microscope

PRODUCTS FEATURES:

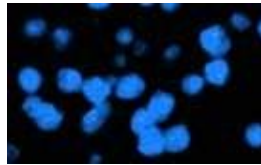
The **OaCP** buffers should be brought to a temperature of 37 °C before use and resuspended to allow optimal mixing of the components.

- **Rapid-ISH Integra** is suggested for "standard samples/probes" (ex. Her-2 FISH tests)
- **Rapid-ISH Integra Plus** is suggested for "difficult samples/probes" (ex. ALK FISH tests)
- **Rapid-ISH Integra Recovery** is suggested for the samples which do not provide valuable results after the first test protocol both with standard and Rapid method
- **Rapid-ISH Hemato** is suggested for "onco-hematological" samples
- **Smart-ISH Solve** is suggested for all type of samples

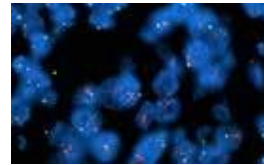
Contains: 1 Vial 100ul (20test)



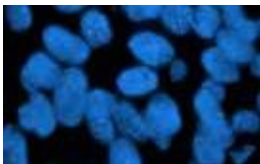
Rapid-ISH
Integra plus
negative
for ALK
rearrangement



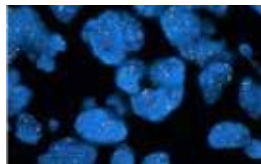
Rapid-ISH Integra
plus, Chromosome
deletion



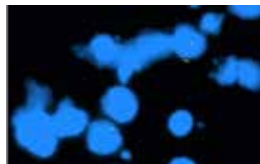
Rapid-ISH Integra,
Her2 gene
amplification



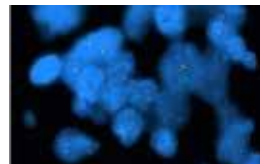
Her-2 before



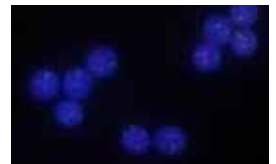
After Rapid-ISH Integra
Plus treatment



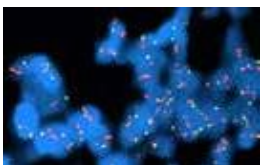
19q before



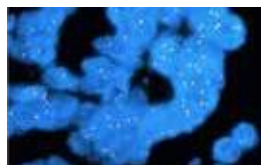
After Rapid-ISH Integra
Plus treatment



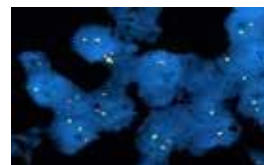
Rapid-ISH Hemato,
P53-CLL



Smart-ISH Solve
Her2 gene
amplification



Smart-ISH Solve
negative for ALK
rearrangement



Smart-ISH Solve
Chromosome
deletion



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All the reagents included in the DoMo Genetics line are protected by current intellectual property laws. Patent granted (Di Oto E., Monti V., Asioli S.)