STANDARD FISH O/N METHOD WITH Smart-ISH Buffer
&
FAST FISH METHOD WITH Rapid-ISH Integra Buffer

FOR THE MAXIMUM PERFORMANCE CHANGE YOUR REAGENT BEFORE THE PROTOCOL START

FOR FFPE TISSUES

Materiali

Xylene or similar solvent for paraffin
Ethanol or similar alcohol mixture at 100% 85%; 70%
Sodium-Citrate Buffer (SSC) 2X pH 7
Citrate buffer pH 8
HCL 0.01N
PepsinE
FISH probes
Rapid-ISH Integra Buffer / Smart-ISH Buffer
Rubber Cement or similar vinyl cement
slide coverslips
Stringency SSC2X / 1.5% NP40 buffer
DAPI counterstain

Instruments

Dry Owen
Water bath
hybridization plate
Coplin Jar

Protocol

Pre-Hybridization steps

- Place the slides in a dry owen at 65 ° C for 30 minutes
- Place in a dry owen at 65 ° C a coplin jar with 50ml of Xylene
- Pre reheated a coplin jar with 50ml of Citrate Buffer pH8 at 98°C in the water bath
- Pre reheated a coplin jar with 50ml of HCL 0.01N at 37°C in the water bath
- Pre reheated a coplin jar with 100ml of SSC2X/NP40 1.5% a 75°C in the water bath

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FAST FISH METHOD WITH Rapid-ISH Integra Buffer

- 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 owen at 65 ° C for 30 minutes
- Proceed with 3 sequential washings of the slides with 50ml of xylene in a coplin at RT for 3 minutes / cad.
- 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 / Cad.
- Dry the slides at RT for 5 minutes
- Incubate the slides in Coplin with the citrate buffer at 98°C for 25 minutes in relation to the characteristics of the sample
- Leave to cool the slides in the same coplin at RT for 10 minutes
- Dissolve 0.250 g of Pepsin in the coplin with HCL at 37 ° C
- Then wash the slides in a quick dip into a Coplin with 50 ml of SSC2X
- Incubate the slides in the Coplin at 37 ° C for about 30 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 / Cad.
- Dry the slides at RT for 5 minutes

Hybridization steps

STANDARD FISH O/N METHOD:

- On each slide affix 3 ul of probe 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 specifications of the probe; Hybridization, temperature according to the specifications of the probe, ***time: o/n***

STANDARD FISH O/N METHOD WITH Smart-ISH Buffer

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FAST FISH METHOD WITH Rapid-ISH Integra Buffer

FAST FISH METHOD:

- On each slide affix 3 ul of probe and 5ul of ***Rapid-ISH Integra Buffer*** (The type of buffer is to be determined in relation to the type of sample to be analyzed; see enclosed data sheets)
- 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 specifications of the probe; Hybridization, temperature according to the specifications of the probe, ***time 40 minutes***

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 / Cad.
- Dry the slides at RT for 5 minutes
- Affix 5-10 ul of DAPI on each slide, cover with coverslip
- Ready for the observation under the microscope