FAST FISH METHOD WITH Rapid-ISH Integra Buffer

FOR THE MAXIMUM PERFORMANCE CHANGE YOUR REAGENTS BEFORE THE PROTOCOL START

FOR FFPE TISSUES

Materials

- •Xylene or similar solvent for paraffin
- • Ethanol or similar alcohol mixture at 100% 85%; 70%
- • Sodium-Citrate Buffer (SSC) 2X pH 7
- • Proteinase K 20mg / ml
- • probe for FISH
- • Buffer Rapid-ISH Integra
- • Rubber Cement or similar vinylic cement
- • Coverslips
- • SSC2X stringency buffer / 1.5% NP40
- • 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 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 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

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- 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 2X SSC at 77 ° C for a time to be determined between 12 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 12 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 / Cad.
- Dry the slides at RT for 5 minutes

Hybridization steps

- 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