STANDARD FISH O/N METHOD FOR CYTOLOGICAL SAMPLES WITH *Smart-ISH Buffer*

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FAST FISH METHOD FOR CYTOLOGICAL SAMPLES WITH *Rapid-ISH Integra Buffer*

FOR THE MAXIMUM PERFORMANCE CHANGE YOUR REAGENT BEFORE THE PROTOCOL START

Materiali

Xylene or similar solvent for paraffin
Ethanol or similar alcohol mixture at 100% 85%; 70%
Sodium-Citrate Buffer (SSC) 2X pH 7
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

If the cytologic preparation has already been colored and mounted with coverglass, place it in a dry owen at 65 °C for 24-48 hours to remove the cover without damaging the material.

- Pre reheated a coplin jar with 50ml of SSC 2X pH 7 at 73°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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- Proceed with 3 sequential washings of the slides with 50ml of xylene in a coplin at RT for 5 minutes / cad. **(Only for mounted and colored cytological samples)**
- 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 SSC 2X pH 7 at 73°C for 3 minutes in relation to the characteristics of the sample **(Only for mounted and colored cytological samples)**
- Dissolve 0.50 g of Pepsin in the coplin with HCL at 37 ° C
- Incubate the slides in the Coplin at 37 ° C for about 25-30 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***

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.***

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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