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 STARTS

Materials

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 Oven
Water bath
hybridization plate
Coplin jar

Protocol

Pre-Hybridization steps

If the cytologic preparation has already been stained and mounted with coverglass, place it in a dry oven 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
- Proceed with 3 sequential washing of the slides with 50ml of xylene in a coplin at RT for 5

minutes/each. **(Only for mounted and stained cytological samples)**

- Dry the slides at RT for 5minutes
- Dehydratetheslidesin2sequentialstepsincoplinwith50mlof100%ethanolfor5 minutes/each.
- Dry the slides at RT for 5minutes

- Incubate the slides in coplin with SSC2X pH7 at 73°C for 3 minutes in relation to the features of the sample **(Only for mounted and stained 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 features of the sample
- Then wash the slides in a quick dip into a coplin with 50 ml ofSSC2X
- Dehydratetheslidesin3sequentialstepsinacoplinwith50mlofEthanol70%-85%-100%for1 minute /each.
- Dry the slides at RT for 5minutes

Hybridization steps

STANDARD FISH O/N METHOD:

- On each slide affix 3 ul of probe and 5ul of ***Smart-ISHBUFFER***
- Cover the area with a coverslip 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 analysed; see enclosed datasheets)
- Cover the area with a coverslip 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 40minutes.***

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 3minutes
- quickly wash slides in a coplin with 50 ml of SSC2X at RT
- Dehydratetheslidesin3sequentialstepsinacoplinwith50mlofEthanol70%-85%-100%for1 minute /each.
- Dry the slides at RT for 5minutes
- Affix 5-10 ul of DAPI on each slide, cover with coverslip
- Ready for the observation under the microscope