

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

&

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

FOR THE MAXIMUM PERFORMANCE CHANGE YOUR REAGENT BEFORE THE
PROTOCOL STARTS

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
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

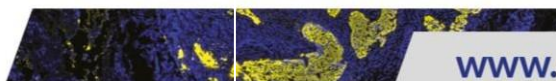
Instruments

Dry Oven
Water bath
hybridization plate
Coplin Jar

Protocol

Pre-Hybridization steps

- Place the slides in a dry oven at 65 ° C for 30minutes
- Place in a dry oven at 65 ° C a coplin jar with 50ml of Xylene
- Preheat a coplin jar with 50ml of Citrate Buffer pH8 at 98°C in the water bath
- Preheat 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



- On the hybridization plate set the fixed temperature at 75 °C
- Incubate the slides on the plate at 75 °C for 5minutes,
- Immerse the slides in Xylene in oven at 65 °C for 30minutes
- Proceed with 3 sequential washing of the slides with 50ml of xylene in a coplin at RT for 3 minutes / each.
- Dry the slides at RT for 5minutes
- Dehydrate the slides in 2 sequential steps in coplin with 50ml of 100% ethanol for 5 minutes/each.
- Dry the slides at RT for 5minutes
- Incubate the slides in Coplin with the citrate buffer at 98°C for 25 minutes in relation to the features of the sample
- Leave to cool the slides in the same coplin at RT for 10minutes
- 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 features 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 50ml of Ethanol 70%-85%-100% for 1 minute /each.
- Dry the slides at RT for 5minutes

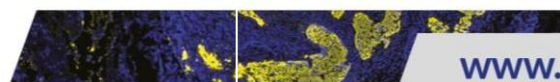
Hybridization steps

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
- Dehydratetheslidesin3sequentialstepsinacoplinwith50ml ofEthanol70%-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

