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 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 for3 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
- IncubatetheslidesinCoplinwiththecitratebufferat98°Cfor25minutesinrelationtothe 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 ofSSC2X
- 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 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

OaCP IE LTD Phoenix House, Monahan Road, T12H1XY, Cork (IE).
Tel IE line: +353 (0)212376197
Tel IT Line: +39 051 021 8095 – **FAX IT Line**: +39 051 021 1389
Mail: ask@oacp.it – **Web**: www.oacp.it
VAT: IE3518703DH; VAT: IT 03537481206