FFPE Tissue Slide Preparation and Hybridization

Required Reagents Supplied

0.01N HCL
10mM Citric Acid (Citric Acid, Tri sodium salt, pH ~6.8)
Pepsin 20mg
2X SSC

Required Reagents & Equipment (Not Supplied)

Xylene
Flow Hood
70% and 100% Ethanol
Water Bath
Humidity Control Cards in dH_2O
Wash Buffer 1- at 73°C
Wash Buffer 2- at room temperature
$22mm^2$ coverslip
22x50 mm coverslip
Thermobrite
DAPI with Antifade

Prep: Heat 10mM Citric Acid (Pre-Treatment Buffer) to 96-99°C in a water bath; Warm 40mL 0.01N HCL to 37°C in water bath; Heat oven to 90°C; Prepare 1mL of 20mg/mL Pepsin stock in H_2O; Prepare 40mL of 2xSSC at room temperature. Prepare 2 separate containers of fresh Xylene and place them in the flow hood. Prepare 3 separate containers of 100% Ethanol and 1 separate container of 70% Ethanol. Soak Humidity Control Cards in dH_2O.

1. **Deparaffinization**
    a. Age slide at 90°C for 20 minutes in oven.
    b. Immerse slides in Xylene for 5 minutes. Repeat with fresh Xylene once. Keep Xylene in flow hood.
    c. Immerse slides in 100% Ethanol for 2 minutes. Repeat once.
    d. Air dry or dry on slide warmer.
2. **Pretreatment**
    a. Place slide in 96-99°C citric acid pretreatment buffer (pH ~6.8) for 30-60 minutes.
        i. Time is variable depending on tissue type and size.
        ii. Lab running the kit will be the determiner on time.
    b. Wash slide in 2x SSC for 2 minutes.
3. **Digestion**

a. Add 1mL of 20mg/mL Pepsin solution to 40mL of pre-warmed, 37°C 0.01N HCL and mix well.
b. Place slide in 37°C pepsin solution for 20-40 minutes.
    i. Time is variable depending on tissue type and size.
    ii. Lab running the kit will be the determiner on time.
c. Wash slides in 2x SSC for 1-2 minutes.
d. Immerse slides in 70% Ethanol for 30 seconds.
e. Immerse slides in 100% Ethanol for 30 seconds.
f. Air dry slide or place on slide warmer.
g. View digestion under microscope. If adequate digestion is not achieved, repeat steps b-f but in 5-minute pepsin increments.
h. If adequate digestion is achieved, proceed to hybridization protocol.

4. Hybridization

a. Prepare 10 µl of probe mix per cellular area (2 µl of probe + 8 µl of buffer).
b. Pipette the probe on the processed tissue slide on top of the cellular area and place a 22x22 mm coverslip on that area, taking care that there are no air bubbles.
c. Seal the coverslip with rubber cement; ensure all edges of the coverslip are sealed with the rubber cement.
d. Place pre-soaked Humidity Control Cards in HYBrite/ ThermoBrite. Hybridize the slides on the HYBrite/ ThermoBrite with the following program:

Denaturation: 75°C for 7 minutes → Hybridization 37°C for 16-24 hours

e. After incubation period, carefully remove the rubber cement and coverslip. Proceed to wash steps.
f. Post hybridization wash step 1: Place slide in Wash Buffer 1 (73° C) for 2 minutes, agitating vigorously for the first 10-15 seconds.
g. Post hybridization wash step 2: Place slide in Wash Buffer 2 (room temperature) for 2 minutes, agitating vigorously for the first 10-15 seconds.
h. Wipe the back of the slide dry and allow the rest of the slide to fully dry in the dark. Counterstain with DAPI with Antifade and coverslip with 22x50 mm coverslip. Allow the slide to sit in the dark for 15-20 minutes before viewing under the microscope.