

# >>FISH Procedure for FFPE Section

## 1. Pre-experiment Preparation

- 1.1. Heat the section slides at 65°C for over 2 hours to overnight or at 80°C for 30 minutes.
- 1.2. Prewarm 40ml of pepsin reaction solution (0.01M HCl) in a Coplin jar placed in a 37°C water bath. Just before use, add 400 µl of 10% pepsin stock solution and mix thoroughly. (Preparation of 10% Pepsin Stock Solution: Dissolve 0.5g pepsin powder with 5ml distilled water, gently mix well, aliquot and store at -20°C. )

## 2. Pretreatment

- 2.1. Dewax the slides by immersing them in xylene or turpentine for 10 minutes, and repeat the process once.
- 2.2. Immerse the slides in absolute ethanol for 5 minutes, and repeat once.
- 2.3. Sequentially immerse the slides in 90% ethanol, 70% ethanol, and distilled water for 2 minutes each.
- 2.4. Place the slides in distilled water and heat-treat them in a water bath at 98-100°C for 20 minutes.
- 2.5. Digest the sections in prewarmed pepsin working solution at 37°C for 10-30 minutes (time variable depending on the sample).
- 2.6. Sequentially immerse the slides in distilled water, 70% ethanol, 90% ethanol, and 100% ethanol for 2 minutes each.
- 2.7. Allow the slide to air dry completely at room temperature.

## 3. Denaturation and Hybridization

- 3.1. Equilibrate the probe to room temperature from -20°C, **vortex to mix thoroughly and centrifuge briefly.**
- 3.2. Apply 10µl of the probe onto the sample, cover with a coverslip, ensuring no air bubbles are trapped, seal the edges with rubber cement.
- 3.3. Denature the slides at 83°C for 5 minutes, followed by hybridization at 42°C for 2 to 72 hours (program set to room temperature after 16 hours).

## 4. Post-wash and Counterstain

- 4.1. Prewarm Wash Buffer II (0.1% Tween-20/0.4xSSC, pH8.0±0.2 ) in a 68°C water bath.
- 4.2. Carefully remove the rubber cement and coverslip using forceps.
- 4.3. Wash the slides with Wash Buffer I (0.1% Tween-20/2xSSC, pH7.2±0.2) at room temperature for 5 minutes.
- 4.4. Perform a gentle wash in prewarmed Wash Buffer II at 68°C for 3-5 minutes.
- 4.5. Wash the slides in 2xSSC at room temperature for 2 minutes.
- 4.6. Sequentially immerse the slides in 70% ethanol, 90% ethanol, and 100% ethanol for 1 minute each.
- 4.7. Allow the slides to air dry completely.
- 4.8. Apply 10 µl of DAPI (with antifade) counterstain solution onto the sample, and cover with a coverslip.