

# >>FISH Procedure for Cell Specimen Slides

## 1. Pre-experiment Preparation

- 1.1. Pipette 3-10 $\mu$ l of cell suspension onto a clean slide. Incubate the slide at 37°C overnight or at 56°C for 30 minutes.
- 1.2. Prewarm 40ml of pepsin reaction solution (0.01M HCl) in a 37°C water bath. Just before use, add 8 $\mu$ l (1.6 $\mu$ l for metaphase cells) 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. Immerse the slide in 2xSSC solution at room temperature for 5 minutes.
- 2.2. Digest the sample in the prepared pepsin working solution at 37°C for 10 minutes.
- 2.3. Rinse the slide in 2xSSC for 5 minutes.
- 2.4. Fix the sample (skip for metaphase cells) by immersing the slide in 1% paraformaldehyde solution for 10 minutes (20-25 minutes for urine sediment cells).
- 2.5. Rinse again in 2xSSC for 5 minutes.
- 2.6. Sequentially dehydrate the slide by immersing it in 70%, 90%, and 100% ethanol, 2 minutes in 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 10 $\mu$ l 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 III (0.1% Tween-20/0.1xSSC, pH8.0 $\pm$ 0.2) in a 68°C water bath.
- 4.2. Carefully remove the rubber cement and coverslip using forceps.
- 4.3. Rinse the slide in 2xSSC for 5 minutes.
- 4.4. Rinse in Wash Buffer III at 68°C for 2 minutes (5 minutes for urine sediment cells).
- 4.5. Rinse again in 2xSSC for 2 minutes.
- 4.6. Dehydrate the slide by immersing it sequentially in 70%, 90%, and 100% ethanol, 2 minutes in each.
- 4.7. Allow the slides to air dry completely.
- 4.8. Apply 10 $\mu$ l of DAPI (with antifade) counterstain solution onto the sample, and cover with a coverslip.