FFPE Pre-Treatment Kit

SKU: **FPTK-001**

Intended Use:
For Research Use Only

Application:
For use in Deparaffinization, Pretreatment, and Digestion of Formalin-Fixed Paraffin Embedded (FFPE) tissues in preparation for fluorescent in situ hybridization (FISH) and chromogenic in situ hybridization (CISH).

Composition:

Component	Concentration	Size
HCL	0.01N HCL	250ml
Citric Acid	10Mm Citric Acid	250ml
Saline Sodium Citrate Buffer	2X SSC	250ml
Pepsin	Not Applicable	5 x 20mg

Storage, Handling, Shelf Life, and Disposal:
Store product excluding Pepsin at 15-30°C, Store Pepsin at -15-20°C; avoid frequent fluctuations in temperature. Expiration date noted on product. Gloves and other protective equipment should be used when handling the kit. Dispose as per local regulations.

Warning and Precaution:
Product does not contain any human or animal components. See Safety Data Sheet (SDS) for more detailed safety and handling information. Do not use expired kit. Avoid cross-contamination. Read instructions for use in full before use.

Limitations:
This product is for research use only.
Performance is dependent on sample preparation, sample quality, and proper storage.
This product is only for use by trained laboratory professionals.

Contact and Support:
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Reagent(s) Not Provided:
- Xylene or Xylene Substitute
- Ethanol
- Wash Buffer 1
- Wash Buffer 2

Equipment Not Provided:
- Flow Hood
- Coplin Jars
- Water Bath
- Humidity Control Cards in dH_2O
- 22x22mm Glass Coverslip
- Lint-Free Wipe
- Timer
- Pipettes (P10)
- Minifuge
- Vortex

FFPE Pre-Treatment Protocol:

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 Pepsin stock in 1mL dH2O; 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 dH2O.

Deparaffinization

1. Age slide at 90°C for 20 minutes in oven.
2. Immerse slides in Xylene or Xylene Substitute for 10 minutes. Repeat with fresh Xylene or Xylene Substitute once. Keep Xylene in flow hood.
3. Immerse slides in 100% Ethanol for 2 minutes. Repeat once.
4. Air dry or dry on slide warmer.

Pretreatment

1. Place slide in 96-99°C citric acid pretreatment buffer (pH ~6.8) for 30-60 minutes.
    a. **Time is variable depending on tissue type and size.**
    b. **Lab running the test will determine the time needed to digest tissues.**
2. Wash slide in 2x SSC for 2 minutes.

Digestion

1. Add 1mL of 20mg/mL Pepsin solution to 40mL of pre-warmed, 37°C 0.01N HCL and mix well.
2. Place slide in 37°C pepsin solution for 20-40 minutes.
    a. **Time is variable depending on tissue type and size.**
    b. **Lab running the test will determine the time needed to digest tissues.**
3. Wash slides in 2x SSC for 1-2 minutes.
4. Immerse slides in 70% Ethanol for 30 seconds.
5. Immerse slides in 100% Ethanol for 30 seconds.
6. Air dry slide or place on slide warmer.
7. View digestion under microscope. If adequate digestion is not achieved, repeat steps 2-7 but in 5-minute pepsin increments.
8. If adequate digestion is achieved, proceed to hybridization protocol.

Hybridization

1. Prepare 10 µl of probe mix per cellular area (2 µl of probe + 8 µl of buffer).
2. 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.
3. Seal the coverslip with rubber cement; ensure all edges of the coverslip are sealed with the rubber cement.
4. 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
5. After incubation period, carefully remove the rubber cement and coverslip. Proceed to wash steps.
6. Post hybridization wash step 1: Place slide in Wash Buffer 1 (73° C) for 2 minutes, agitating vigorously for the first 10-15 seconds.
7. Post hybridization wash step 2: Place slide in Wash Buffer 2 (room temperature) for 2 minutes, agitating vigorously for the first 10-15 seconds.
8. 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.