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Alternative Pretreatment To Product CL-003

Tissue Sample Pretreatment Instructions

SOLUTIONS FOR RESEARCH & ADVANCED DIAGNOSTICS

1. Sample: Tissue

- Paraffin-embedded tissue samples fixation with 4% formaldehyde for 6-72 hours. Slides thickness: 3-4μm.
- Glass slides: Adhesive slides.

2. Pretreatment procedure: Tissue

- Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.
- Dewaxing: According to the customer laboratory protocol (Commonly with Xylene or Limonene for 15min).
- Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.
- Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying.
- Permeation: Immerse the slides in deionized water at 100°C and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.
- Digestion: Protease enzymic digestion at 37°C for 10-40 minutes. Mix the protease work buffer (50mmol HCl) and the 10x protease solution (Pepsin concentration 5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.
- Washing: Wash with 2xSSC at room temperature for 5 minutes.
- Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time. Remove the excess of EtOH solution on the slides by air-drying.

3. Denaturation and Hybridization

- Before the probe usage, centrifuge for 5 seconds to mix well.
- Place the slides in the hybridizer, denature at 85°C for 5 minutes (the hybridization instrument should be preheated to 85°C), and hybridize at 42°C for 2h.

4. Post-hybridization Washing

***Note**: Perform the following steps in a dark room.*

- Take out the hybridized slides, remove the rubber glue, immerse the slides in 2xSSC for 5 seconds, and then gently peel off the coverslip with tweezers.
- Wash the slides in 2xSSC at room temperature for 1 minute.
- Take out the slides and immerse them in 0.3% NP-40/0.4xSSC solution preheated at 68°C for 2 minutes (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2).
- Take out the slides, immerse it in deionized water at 37°C for 1 minute, and then dry the slides naturally in a dark place.
- Drop 10μL of DAPI counter-stain on the hybridization area and cover with a coverslip immediately.

[Method Limits]
This method is only applicable to the pretreatment of neutral formalin-fixed paraffin-embedded tissue sections by fluorescence in situ hybridization. Do not apply to other methods of fixed and embedded tissue sections.

[Instruction version and approval date]
Manual version: V1.0
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Alternative Pretreatment to Product CL-003

Cells Sample Pretreatment Instructions

1. Sample: Cells

- Anticoagulated fresh bone marrow (storage conditions 4°C within 24h). Bone marrow cells accurate fixation (storage conditions -20°C within 6 months).
- Glass slides: Ordinary or common glass slides.

2. Pretreatment procedure: Cells

- Baking: Slides heating at 56°C for 30min or overnight at room temperature.
- Washing: Wash with 2xSSC at room temperature for 5 minutes.
- Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time.
- Remove the excess of EtOH solution on the slides by air-drying.

3. Denaturation and Hybridization

- Take out the probe, put it sill at room temperature for 5 minutes, mix the probe upside down, centrifuge briefly, and drop 10μL of the probe into the hybridization area on the slide. Immediately cover with a 22mm×22mm coverslip. The probe solution should spread out evenly without air bubbles under the coverslip, and then seal the edge with rubber glue (the edge must be completely sealed to prevent water evaporation during the hybridization and affect the detection result).
- Place the slides in the hybridizer, denature at 88°C for 2 minutes (the hybridization instrument should be preheated to 88°C), and hybridize at 45°C for 2h.

4. Post-hybridization Washing

***Note**: Perform the following steps in a dark room.*

- Take out the hybridized slides, remove the rubber glue, immerse the slides in 2xSSC for 5 seconds, and then gently peel off the coverslip with tweezers.
- Wash the slides in 2xSSC at room temperature for 1 minute.
- Take out the slides and immerse them in 0.3% NP-40/0.4xSSC solution preheated at 68°C for 2 minutes (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2).
- Take out the slides, immerse it in deionized water at 37°C for 1 minute, and then dry the slides naturally in a dark place.
- Drop 10μL of DAPI counter-stain on the hybridization area and cover with a coverslip immediately.

[Method Limits]
This method is only applicable to the pretreatment of cells sample by fluorescence in situ hybridization. Do not apply to other methods of cells sample.

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