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Fast FISH Technology Leader

FastProbe®

Introduction to the Fast Fluorescence In-Situ Hybridization (FISH) Experiment Process

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Your Authentic Partner in Molecular Cytogenetics

Sample types and requirements

- **Tissue sample**
  - Surgical tissue section
  - Puncture tissue section
  - Cell mass

- **Fixation:** 4% neutral formaldehyde fixation for 6-48h
- **Slides thickness:** 3-4μm
- **Slides:** Adhesive slides

Tissue sample pretreatment

- **Baking** : 80°C for 30min or 65°C for 2h above.
- **Dewaxing** : 68°C dewaxing agent 15min.
- **Washing** : Anhydrous ethanol at room temperature for 5min.
- **Permeation:** 90°C permeabilizing agent 20min.
- **Washing:** 37°Cdeionized water for 3min.
- **Digestion:** 37°C enzyme working solution for 10-40min.
- **Washing:** Rinse 2 times at room temperature with the washing solution, 5min each time.
- **Dehydration:** 70%, 85% and 100% gradient ethanol at room temperature for 2 minutes each.
- **Drying:** dry at room temperature.

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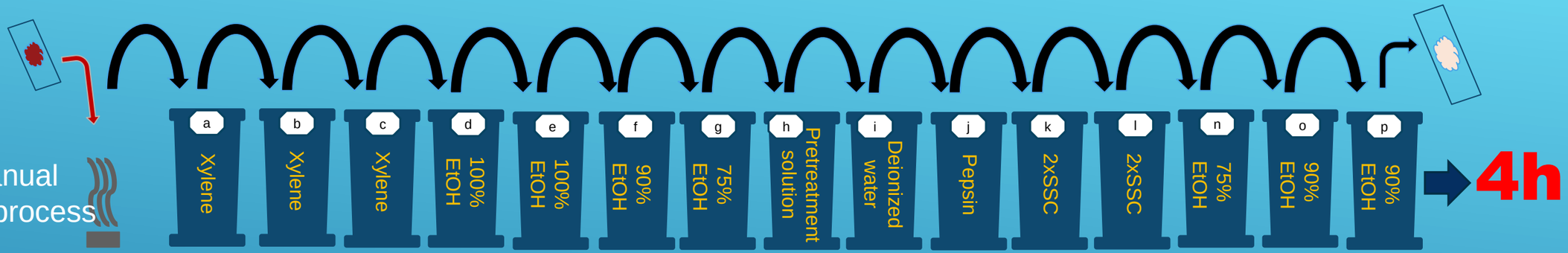
Pretreatment

Traditional manual pretreatment process



Baking 65*C, 2h or Overnight

a-b-c: Dewaxing at room temperature 10min each time.

d: Washing at room temperature 10min.

e, f, g: Washing at room temperature 3min.

h: Pretreatment at 80*C, 20min.

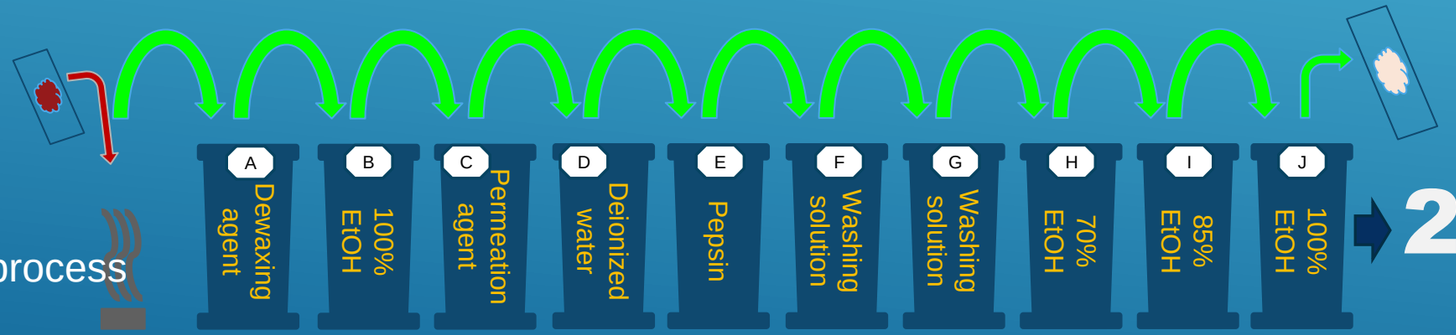
i: Pretreatment at room temperature 3min.

j: Digestion at 37*C, 10-30min.

k, l: Wash at room temperature 5min.

n, o, p: Dehydration at room temperature 2min.

FastProbe® pretreatment process



Uses eco-friendly dewaxing agent

Baking 80*C, 0.5h

A: Dewaxing at 68*C, 15min.

B: Washing at room temperature 5min.

C: Permeation at 90*c, 5min.

D: Washing at 37*C, 3min

E: Digestion at 37*c, 10-30min.

F, G: Washing at room temperature, 5min each time.

H, I, J: Washing at room temperature, 2min each time.

Principles and precautions of each step of tissue sample pretreatment

1. Slides baking: 80°C for 30min or 65°C for 2h or more. **This makes the tissue cells adhere tightly and prevent them from falling off during subsequent washing and other treatments.** Baking time at 80°C should not be more than 30 min. long baking time leads to bad or poor cell morphology.

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Principles and precautions of each step of tissue sample pretreatment

2. Dewaxing: Is performed at 68°C for 15min and the dewaxing agent is preheated 30min ahead of time. To ensure sufficient dewaxing effect, dewaxing should start after the temperature reaches 68°C. Our dewaxing agent is eco-friendly, non-toxic and low volatility, low harm risk for the operator, and no need to operate under a fume hood.

3. Washing: Is performed at room temperature with absolute ethanol for 5 minutes. The liquid level of the absolute ethanol should be higher than that of the dewaxing agent to ensure that the dewaxing agent on the slide is washed away to prevent the influence on subsequent experiments.

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Principles and precautions of each step of tissue sample pretreatment

4. Permeation: Is performed at 90°C for 20 minutes, and the permeation agent is preheated 30 minutes in advance. High temperature treatment of the permeation agent can remove protein cross-links, make cells swell and loosen the tissues, and increase cell permeability, which is beneficial to enzyme digestion and probe hybridization.

- **Insufficient permeation temperature and insufficient time lead to the not fully removal of proteins cross-linking, poor cell permeability, affecting the digestion and hybridization. Too long permeation time lead to cell nucleus swelling, cells sticking together, affecting cell count.**

- **Our in house developed pretreatment reagent improve the success rate of one-time detection, avoid retest, save probe waste.**

Principles and precautions of each step of tissue sample pretreatment

5. Washing: Is performed at 37°C for 3 minutes. The liquid level of the deionized water should be higher than that of the permeation agent to ensure that the permeation agent on the glass slide is washed away to prevent the influence on subsequent experiments.

Principles and precautions of each step of tissue sample pretreatment

6. Digestion: Is performed at 37°C with the enzyme working solution for 10-30 minutes. **Preheat the protease working buffer (45ml) at 37°C at the beginning of the experiment, add 10x protease solution (5mL) when the permeation starts, and continue to preheat for 15 minutes before starting the enzyme digestion , to ensure the enzyme maximum activity.**

- The enzyme digestion time is determined according to the slide thickness, generally 20min digestion for 3μm, 25min digestion for 4μm, and the degree of enzyme digestion is determined under the bright field of the microscope. Digestion is to remove proteins between tissues and cells, which is conducive to the probe's hybridization into the nucleus and reduces the background noise.

Principles and precautions of each step of tissue sample pretreatment

7. Washing: Rinse twice at room temperature with washing solution (2xSSC) for 5min each time. Stop the enzyme digestion to prevent the enzyme's influence on subsequent experiments.

8. Dehydration: 70%, 85% and 100% gradient ethanol at room temperature for 2min each time.

9. Drying: Dry at room temperature.

Tissue sample denaturation and hybridization

- Take out the probe from the refrigerator at -20°C, leave it at room temperature for 5 minutes. Turn the probe upside down manually to mix well, and centrifuge briefly (Do not vortex or us any shaking or oscillator device). Take 10μL and drop on the hybridization area of the cell patch on the slide, and immediately cover with a 22mm×22mm lid (cover glass). The probe should be evenly spread under the cover glass slide without bubbles, and the edge should be sealed with rubber (the edge must be completely sealed to prevent the dry slide from affecting the test results during the hybridization process).

- Place the slides on the hybridization machine (hybridizer) and denature at 85°C for 5 minutes (the hybridization instrument should be preheated to 85°C in advance), and hybridize at 42°C for 2 to 16 hours.

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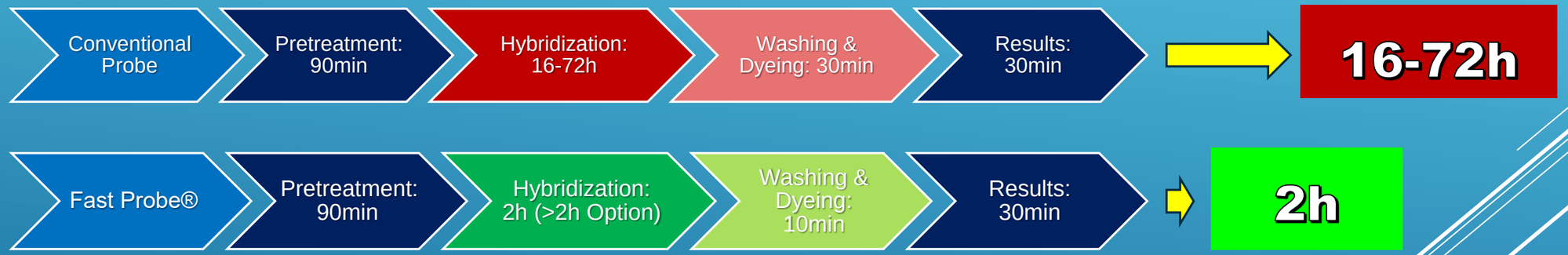
Conventional and HealthCare Biotech (Fast Probe®) Probe Timing Comparison



The world's first manufacturer of 2 hours fast hybridization FISH probe.

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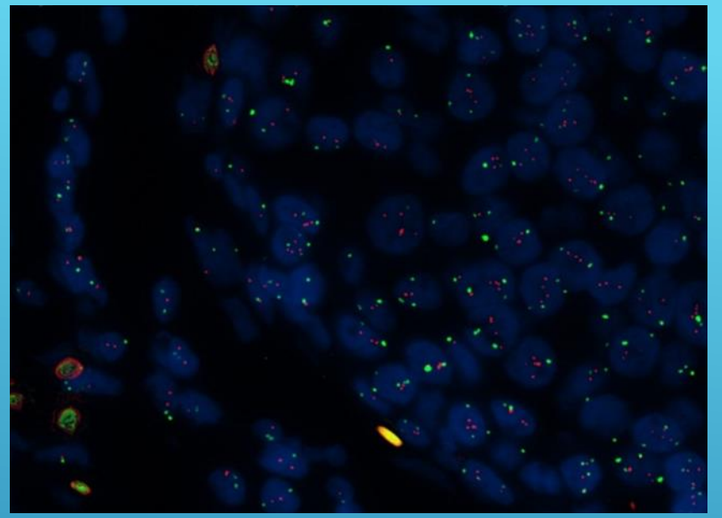
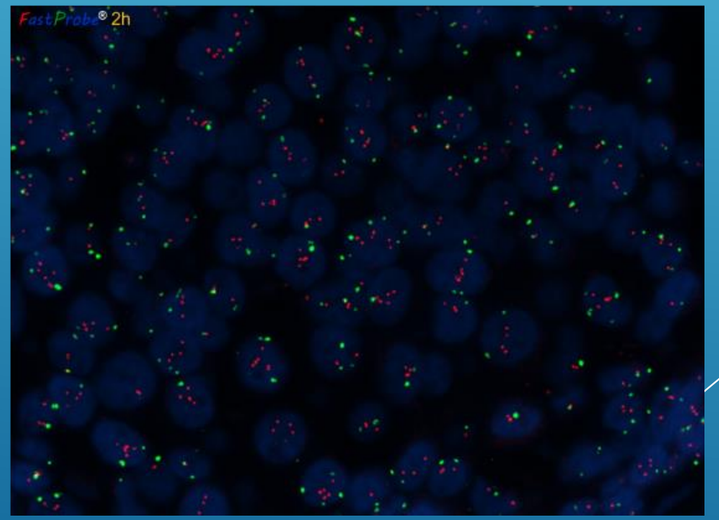
Conventional probe result after
16 hours of hybridization



FastProbe® result after
2 hours of hybridization



Washing and counterstaining of tissue samples post-hybridization

- Use tweezers to carefully remove the sealing glue around the cover glass and the slide. To avoid sticking or moving the cover glass, immerse the slide in 2xSSC solution for about 5 seconds, take it out, and gently push the corner of the cover glass to the edge of the slide with tweezers. Use tweezers to gently peel off the cover sheet.
- Place the slides in **2xSSC and wash at room temperature for 1 min**.
- Take out the slides and immerse in **0.3%NP-40/0.4xSSC solution preheated at 68°C for 2 minutes**.
- Take out the slides and immerse in **deionized water preheated at 37°C for 1 min**. Dry the slides naturally in the dark.
- Drop 10μL of DAPI counterstain on the hybridization area, and cover with the cover slip immediately.

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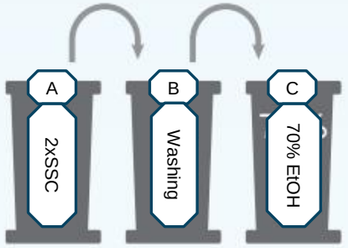
Post-hybridization

Conventional probe

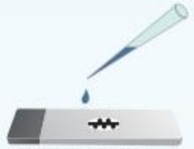
Washing (long) : 18min



Washing

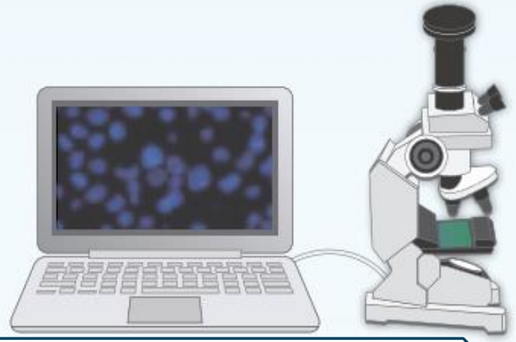
A: 37°C (10min) ; B: 37°C (5min) ; C: Room temperature (3min)

Slide drying



Add 10μL DAPI

Cover slip

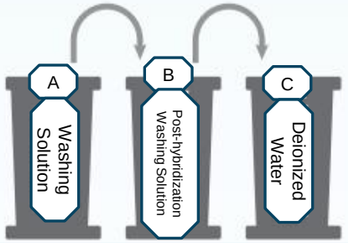


Result observation under fluorescence microscope

Cleaner background and result easy interpretation

FastProbe®

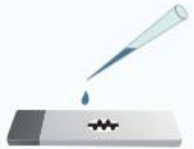
Washing (short) : 4min



Washing

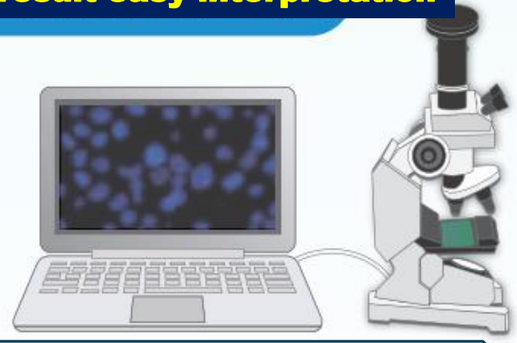
A: Room temperature (1min) ; B: 68°C (2min) ; C: 37°C(1min)

Slide drying



Add 10μL DAPI

Cover slip

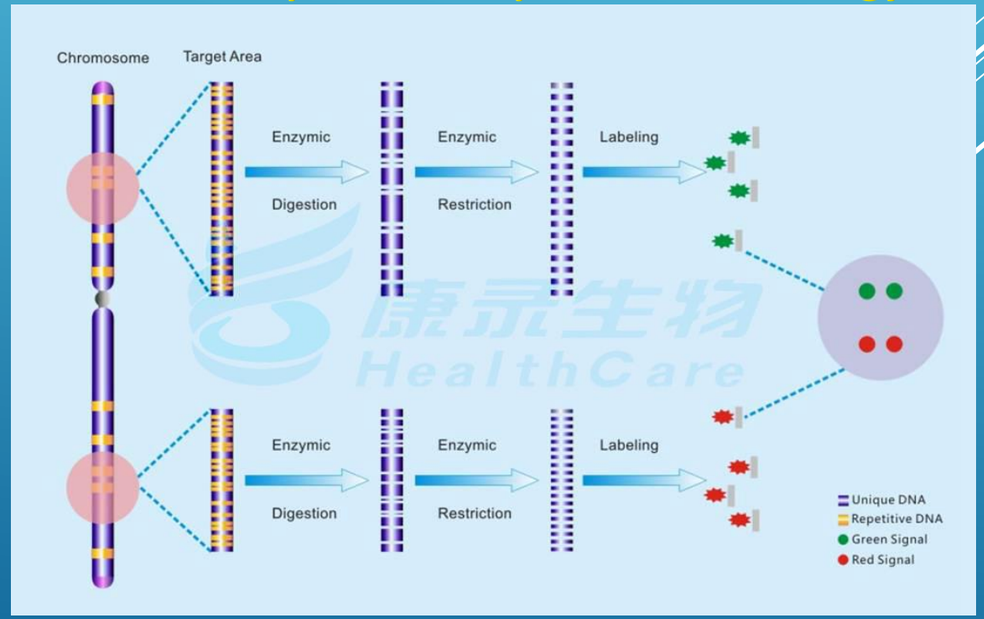


Result observation under fluorescence microscope

HealthCare Biotech Fast FISH Probe Characteristics

- Hybridization time is only 2 hours.
- Higher specificity and sensitivity than traditional FISH probes.
- Good anti-quenching effect.
- Fast FISH probe (FastProbe®) is built on Non-Repetitive Sequences Technology.

The Non-repetitive Sequences Technology



Fast FISH experiment process summary and explanation

1. PRETREATMENT

A. Tissue - Slides pretreatment

1. Slides baking: Bake slides at 80°C for 30min.
2. Dewaxing: Immerse slides in preheated 68°C dewaxing agent for 15 minutes.
3. Slides washing: Take out the slides and immerse in 100% ethanol for 5 minutes at room temperature.
4. Permeation: Take out the slides and immerse in the preheated 90°C permeabilizing agent for 20 minutes.
5. Slides washing: Take out the slides and immerse in preheated 37°C deionized water for 3 minutes.
6. Enzymatic digestion: Take out the slides and immerse in preheated 37°C protease working solution for 10-40 minutes. (Protease working solution preparation: Enzyme working buffer and Protease solution (10x) in 9:1 proportion, or 0.5g Protease powder in 100mL Enzyme working buffer. Take 10x protease solution, shake well, dilute with protease working buffer, and mix well evenly. The protease-working buffer should be preheated at 37°C before preparation. The protease working solution is prepared for current use; discard the solution after one use).
7. Slides washing: Take out the slides, soak in washing solution (2xSSC) and rinse twice for 5 minutes each time.
8. Dehydration: Take out the slides and immerse in 70%, 85% and 100% gradient ethanol for 2 minutes each time.
9. Slides drying: Take out the slides and dry at room temperature, then start the hybridization step process.

B. Cell - Slides pretreatment

1. Slides baking at 56°C for 30min.
2. Slides washing: At room temperature with the washing solution twice, 5min each time.
3. Dehydration: With 70%, 85% and 100% gradient EtOH for 2min each time.
4. Slides drying: Dry at room temperature before hybridization step.

Fast FISH experiment process summary and explanation

2. DENATURATION & HYBRIDIZATION

A). Take out the probe and keep for 5 minutes at room temperature. Manually mix the probe upside down and centrifuge briefly. Take 10μL and drop on the hybridization area, cover immediately with a 22mm×22mm cover glass, the probe should be evenly spread under the cover glass with no bubbles. Use rubber glue to seal the edge thoroughly to prevent any leakage or air exchange from affecting the test result during the hybridization process.

B).

Tissue sample: Put the slides on the hybridization instrument, and denature at 85°C for 5 minutes (the hybridization instrument should be preheated to 85°C in advance), and hybridize at 42°C for 2~16 hours.

Cell sample: Put the slides on the hybridization instrument, and denature at 88°C for 2 minutes (the hybridization instrument should be preheated to 88°C in advance), and hybridize at 45°C for 2~16 hours.

Fast FISH experiment process steps explanation

3. WASHING & COUNTERSTAINING

The following operations should be carried out in the dark room.

1. Take out the hybridized slides, remove the rubber glue on the cover glass, immerse the slice in 2xSSC solution for about 5 seconds, and gently peel off the cover sheet with tweezers.

2. Wash the slides at room temperature with 2xSSC solution for 1min.

3. Take out the slides and immerse in the 0.3%NP-40/0.4xSSC solution preheated at 68oC for 2 minutes.

4. Take out the slides and immerse it in 37°C deionized water for 1 min, and dry the slides naturally in the dark.

5. Drop 10μL of DAPI counterstain on the hybridization area, and cover immediately with a cover slip.

4. RESULT OBSERVATION & INTERPRETATION

The target area analysis is performed under fluorescence microscope. HealthCare Biotech FastProbe® filter parameters: Green (495/518), Orange (553/565), DAPI (367/452). Result interpretation is done according to the product instructions.

Fast FISH experiment process steps explanation

1). Slides Baking: The purpose is to prevent the tissue from peeling off, so that the tissue cells tightly adhere to the glass slide, and will not fall off during subsequent washing and other treatments.

Note: The baking time at high temperature should not exceed 30 minutes. Long baking time leads to poor cell morphology. If there is a worry about forgetting to bake, you can use 65°C baking slides for 2h.

2). Dewaxing: Use environmentally friendly dewaxing agent instead of xylene. The reagent has low toxicity and low volatility. It does not need to be carried out in a fume hood. (The dewaxing agent components are Dipentene, limonene, mineral oil).

Note: It is necessary to preheat the dewaxing agent 30 minutes in advance, and start dewaxing after the reagent temperature reaches 68°C to ensure sufficient dewaxing effect.

3). Washing with absolute ethanol: The purpose is to remove residual paraffin and dewaxing agent.

Note: The liquid level of absolute ethanol must be higher than that of the dewaxing agent to ensure that the dewaxing agent on the slide is completely washed.

Fast FISH experiment process steps explanation

4). Permeation: The permeation agent high temperature treatment can remove protein cross-links and expose DNA. The permeabilizing agent makes at the same time tissues soft, cells swell, increases cell membrane permeability, which is conducive to a better enzyme digestion and enhances probe hybridization into the nucleus.

Note: Preheat the permeation agent 30 minutes in advance (if the temperature of the water bath is slower, it will take longer), the permeabilizer temperature should reach 90°C before the permeation beginning. Insufficient permeation temperature and insufficient permeation time lead to the incomplete proteins cross-linking removal, poor cell permeability which affecting the digestion and probe hybridization. If the permeation time is too long, the cell nucleus will swell, and the cell nucleus will stick together affecting the cell count.

5). Deionized water: The pure water aims at washing away the permeation agent on the slides, and balance the slides temperature to 37° C, without affecting the subsequent enzymatic digestion.

Note: The liquid level of the deionized water is higher than that of the permeabilizer to ensure that the permeabilizing agent on the glass slides is washed off.

Fast FISH experiment process steps explanation

6). Enzymic digestion: The purpose of digestion is to remove the proteins between and within the cells, to disperse the cells, and to facilitate probe hybridization.

How to use the enzyme working solution? Preheat the enzyme-working buffer at 37°C at the beginning of the experiment, and immediately after the permeabilization starts, add 10x enzyme solution.

7). 2xSSC washing: This aims to stop the enzyme digestion and wash off the digested products.

Probe hybridization:

The probe is sticky, and red DNA will precipitate in the upper layer after long-term freezing. It must be fully inverted and mixed before use.

Mixing method: Invert manually the probe at least 5 times till no aggregated red material is seen, and then collect the probe to the bottom of the tube by instantaneous centrifugation.

Fast FISH experiment process steps explanation

Denaturation & hybridization (Reminder):

- **Tissue sample: Total denaturation at 85° C for 5 minutes (hybridization instrument should be preheated to 85ºC in advance), hybridization at 42ºC for 2 hours.**
- **Cell sample: Total denaturation at 88ºC for 2 minutes (hybridization instrument should be preheated to 88ºC in advance), hybridization at 45ºC for 2 hours.**

Washing & Counterstaining after hybridization:

- **Tear the adhesive: Use tweezers to carefully remove the sealing glue around the cover glass to avoid sticking or moving the cover glass.**
- **2xSSC washing: Immerse the slides in 2xSSC for about 5 seconds, take out, gently push the corner of the cover slip to the edge of the slide with tweezers, gently peel off the cover sheet with tweezers, and place the slide at 2xSSC room temperature for 1min.**
- **The washing solution after hybridization is 0.3%NP-40/0.4xSSC, must be preheated 30min in advance. It is colorless and transparent at room temperature. The reagent will become turbid after heating up. The washing time is 2min.**
- **Washing with deionized water aims at washing off the post-hybridization solution on the slides. If dried directly, the slides will be dirty.**
- **Before DAPI addition, the tissue must be fully dried. The whitening of the tissue is considered as tissue complete drying, otherwise, the signal is easily allayed.**

Thanks for having your attention.