Frozen Tissue Preparation

Notes

- The pretreatment process pre-treats the sample prior to denaturation and hybridization with the appropriate probes. Following pretreatment, the slides and appropriate probes are denatured; the slides are hybridized, washed and counterstained prior to analysis.
- Some tissues are not appropriate for FISH analysis.
- Further optimization of the protocol may be required.

Required Reagents & Equipment (Not Supplied)

0.01N HCl
Fixative – 3:1 Methanol : Acetic Acid
10 mM Citric Acid (Citric Acid, Tri sodium salt, pH ~6.8)
Pepsin Sigma P7012-5G - Prepare 80 mg/ml stock in H_2O
70%, 85%, 100% Ethanol

Protocol

1. Pre-warm 40 ml 0.01N HCl in a jar in 37°C water bath.
2. Optional: Pre-warm a jar with 10 mM Citric Acid to 80°C in a water bath.
3. Cut 5 µm sections on silanized or positively charged slides.
4. Dry at room temperature for 15-30 minutes.
5. Fix in fixative (3:1 methanol : acetic acid) for 5 minutes.
6. Optional: Place slide into Citric Acid jar for 55 minutes.
7. Add 1 ml of Pepsin (see above) to 40 ml 0.01N HCl from step 1. Different tissues may require a different Pepsin concentration.
8. Place slide into Pepsin solution for 30 minutes. Pepsin concentration and incubation time may require optimization.
9. Rinse slide in 70% Ethanol for 30 seconds.
10. Air-dry slide and check slide for proper digestion. There should be dark, distinguishable cells.
11. Dehydrate slide though 70%, 85% and 100% Ethanol, each for 2 minutes.
12. Air dry slide and proceed to hybridization.

References

Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual, Third Edition. Lippincott-Raven Philadelphia. 1991.

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