Frozen Tissue Preparation

Required Reagents & Equipment (Not Supplied)

0.01N HCl
Methanol
Acetic Acid
Pepsin (Sigma P7012-5G)
70%, 85%, 100% Ethanol
Coplin jars
Water bath

Preparation

1. Pre-warm 40 ml 0.01N HCl in a Coplin jar in a 37°C water bath.
2. Prepare Carnoy's fixative by mixing 3 parts methanol to 1 part acetic acid. Put the prepared fixative into a 2-8°C fridge for at least 1 hour prior to use.
3. Prepare 80 mg/ml pepsin stock in H_2O.

Protocol

1. Cut 3-5 µm sections on positively charged slides or slides pre-coated with Silane.
2. Dry at room temperature for 15-30 minutes.
3. Fix in ice-cold Carnoy's fixative for 10 minutes.
4. Add 1 ml of prepared pepsin (see above) to the pre-warmed 0.01N HCl.
5. Place slide into pepsin solution for 10 minutes.
6. Rinse slide in 70% Ethanol for 30 seconds.
7. Air dry slide and check slide for proper digestion. There should be dark, distinguishable cells.
8. Dehydrate slide though 70%, 85% and 100% Ethanol, each for 2 minutes.
9. Air dry slide and proceed to hybridization.

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. Please refer to the hybridization protocol for more details.
- Further optimization of the protocol may be required; pepsin concentration and incubation time may require optimization, and different tissues may require a different pepsin concentration.
- Carnoy's fixative should be prepared fresh each day it is used.

References

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