

Fixation of Cytological Smears, Fine-Needle Aspirates, and Touch Preparations

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Ethanol, absolute

RNase A

Boehringer, Mannheim, Cat. 109169 (100 mg)

SSC, 2X

Preparation

RNase A

Stock solution: 20 mg RNase A/ml sterile water

Boil for 15 min

Cool to room temperature

Aliquot and store at -20°C

Procedure

1. Air dry preparations. Air-dried slides can be stored in the refrigerator.
2. Soak slides gently and briefly in 2X SSC.
3. Incubate slides in RNase A solution (1:200 in 2X SSC) in a Coplin jar at 37°C for 20 min. Do not agitate slides more than necessary (danger of losing cells).
4. Rinse gently in 2X SSC (twice).
5. Fix for 3 min in 70% ethanol, for 3 min in 90% ethanol, and for 10 min in 100% ethanol.
6. Air-dry. Proceed with FISH-pretreatment protocol (leave out the RNase pretreatment).

7. Alternatively, air-dried slides can be fixed directly in ethanol (step 5) and the RNase treatment can be done afterwards. (Danger of losing cells is less but background fluorescence can be higher).