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Guidelines for *In Situ* Hybridization

An *in situ* hybridization protocol follows this general outline:

- Preparation of slides and fixation of material
- Pretreatments of material on slides, *e.g.*, permeabilization of cells and tissues
- Denaturation of *in situ* target DNA (not necessary for mRNA target)
- Preparation of probe
- *In situ* hybridization
- Posthybridization washes
- Immunocytochemistry
- Microscopy

All these steps are discussed below in detail. The information provided will make it easier to decide whether a certain step should or should not be included in a given *in situ* hybridization protocol.

Details of the Technique

Slide preparation

For chromosome spreads, alcohol/ether (1:1) cleaned slides are sufficient. However, since tissue sections may be lost during the procedure, use either polylysine or glutaraldehyde-activated gelatin chrome aluminum slides for these sections.

Fixation

To preserve morphology, the biological material must be fixed. From a chemical point of view, there is little limitation in the type of fixation used because one of the following will be true:

- The functional groups involved in base pairing are protected in the double helix structure of duplex DNA.
- RNA is fairly unreactive to crosslinking agents.
- The reaction is reversible (*e.g.*, with formaldehyde).

For metaphase chromosome spreads, methanol/acetic acid fixation is usually sufficient. For paraffin-embedded tissue sections, use formalin fixation. Cryostat sections fixed for 30 min with 4% formaldehyde or with Bouin's fixative have been used successfully, as well as paraformaldehyde vapor fixation. Tissues can also be freeze-dried.

It should be noted that the DNA and RNA target sequences are surrounded by proteins and that extensive crosslinking of these proteins masks the target nucleic acid. Therefore, permeabilization procedures are often required.

Unfortunately, a fixation protocol which can be used for all substrates has not yet been described. The fixation and pretreatment protocols must be optimized for different applications.