



FISH on Paraffin-embedded tissues

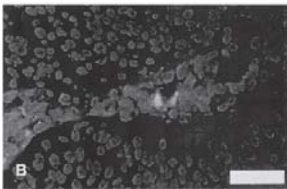
Pre-treatment

Pre-treatment is an important and delicate step of FISH preparations. It consists of a cell/tissue digestion, and it is especially recommended before hybridization step is performed in difficult samples (with a lot of cytoplasmic residues) and in formalin-fixed paraffin embedded tissues. Pre-treatment by tissue digestion enables nucleic acids to be unmasked and then to be easily accessible by FISH probes. This must be achieved with minimal tissue damage, to maintain tissue morphology and cell structure. To do so, it is necessary to test various digestion times and to check tissue sections following proteolytic digestion, to evaluate the slide preparation before proceeding with the hybridization steps.

The figures below show three different examples of pretreatment on a tissue sample



(A) Underdigested tissue section. Although the nuclei can be discerned, protein remains, which masks the preparation that would lead to inefficient hybridization. In this case, we recommend extension of the pepsin treatment to a maximum of 45 minutes.



(B) This represents an optimally digested tissue section. Nuclei are clearly visible.



(C) This tissue section is overdigested, as the morphology is beginning to disappear and the nuclear boundaries become irregular. In this case, we recommend reducing baking time at 56°C and pepsin treatment time.

Images from: Molecular Biotech., 21; 217-220

Poseidon Satellite Enumeration Probes (SE): hybridization in paraffin-embedded tissues

Please remember that our SE probes are provided in a 5x concentrated format. If you would like to use the probes for hybridization in paraffin-embedded tissues, please dilute the probes in PTB Buffer (Paraffin Tissue Hybridization Buffer).