

Enzymatic digestions of Lowicryl ultrathin sections prior to post-embedding hybridization

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Equipment and reagents

- ◆ Gold grids bearing Lowicryl K4M sections
- ◆ Parafilm
- ◆ Incubator at 37°C
- ◆ Tris-HCl buffer M, pH 7.3 stored at 4°C
- ◆ 0.2 mg/ml protease (or 1 mg/ml proteinase K) in distilled water
- ◆ Ribonuclease A (RNase A) (1 mg/ml) in Tris-HCl 10 mM, pH 7.3
- ◆ Deoxyribonuclease I (DNase I) (1 mg/ml) in Tris-HCl 10 mM, pH 7.3, containing 5 mM MgCl₂, 2 mM RNasin ribonuclease inhibitor and 2 mM dithiothreitol
- ◆ S1 nuclease at 1,600 U/ml (stock at 400 U/μl) in 1 x S1 nuclease buffer (10 x S1 nuclease buffer, pH 7.5: 0.33 M sodium citrate, 0.5 M sodium chloride, 0.3 mM zinc sulfate).

Method^a

- 1 Collect ultrathin Lowicryl K4M sections of fixed material (see [Successive steps for Lowicryl K4M embedding and ultrathin sectioning of somatic mammalian cells prior to in situ hybridization](#)) on Formvar carbon coated gold grids (see [Preparation of Formvar carbon coated grids for ultrathin sections of Lowicryl embedded cells prior to in situ hybridization](#)).
- 2 Prepare the enzymatic solutions just before use
- 3 Distribute 10 μl drops of enzymatic solutions on a sheet of Parafilm in a wet chamber.
- 4 Float the grids on the surface of the enzyme-containing drops and put the wet chamber in an incubator at 37 °C for either 15 min with protease^b or 1 h with proteinase K,^b RNase,^c DNase^d and S1 nuclease.^e
- 5 Rinse the grids rapidly at room temperature on three successive drops of distilled water distributed on a Parafilm sheet.
- 6 Wash the grids extensively in a jet of distilled water.
- 7 Air dry the grids.

- 8 The dry digested grids can be stored or immediately processed for hybridization with or without denaturation treatment (see [Post-embedding in situ hybridization and detection of hybrids](#) and [Denaturation of DNA in ultrathin sections of Lowicryl K4M embedded material](#)).

Notes

- a Several nuclease treatments with or without protease pre-treatment can be done successively on the same grid.
- b Protease or proteinase K is used to eliminate the proteins of the biological material which would hide some nucleic acid sequences and/or exhibit interactive binding sites with the probe.
- c When localizing DNA sequences, a prior RNase A digestion is performed in order to prevent concomitant detection of related RNA sequences.
- d When localizing RNA sequences, a prior DNase I digestion is required only in those biological models systems which contain related single-stranded DNA sequences.
- e To detect exclusively double-stranded DNA sequences in model systems containing both single DNA strands and duplex DNA molecules, a prior S1 nuclease digestion is required to eliminate all single-stranded nucleic acid molecules, both RNA and DNA.