

Denaturation of DNA in ultrathin sections of Lowicryl K4M embedded material

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Equipment and reagents

- ◆ Gold grids bearing Lowicryl K4M sections of formaldehyde-fixed material
- ◆ Parafilm
- ◆ 0.5N NaOH stored at room temperature for several months

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 Eliminate the proteins of the sections with either a 15-min 0.2 mg/ml protease or a 1-h 1 mg/ml proteinase K treatment performed at 37°C (see [Enzymatic digestions of Lowicryl ultrathin sections prior to post-embedding hybridization](#)).
- 3 Distribute 10 µl drops of 0.5 N NaOH (prepared just before use from a 5N stock solution) on a sheet of Parafilm.
- 4 Place the grids on the NaOH drops for 4 min at room temperature.
- 5 Rinse the grids by rapid passages on three 10 µl drops of distilled water.
- 6 Wash the grids in a jet of distilled water.
- 7 Air dry the grids for about 10 min.
- 8 Use the grids for *in situ* hybridization (see [Successive steps for specific detection of DNA by post-embedding *in situ* hybridization](#) and [Specific detection of double-stranded DNA in Lowicryl K4M sections by *in situ* hybridization](#)).

Notes

- a Denaturation of the double-stranded DNA molecules in a section is required for localizing double-stranded DNA target molecules. It is omitted for localizing exclusively single-stranded DNA sequences (such as the single-stranded portions which are randomly distributed in the herpes simplex virus genomes and the single-stranded molecules which appear during the replication of adenovirus genomes). Under our experimental conditions, 0.5N NaOH treatment of sections

resulted in an extensive denaturation of double-stranded cellular and viral DNA following formaldehyde fixation of cells (but not glutaraldehyde fixation) and after enzymatic elimination of the proteins of the Lowicryl K4M sections.