

Hybridization solution: composition, heat treatment prior to post-embedding *in situ* hybridization

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Equipment and reagents

- ◆ Boiling water-bath
- ◆ Deionized formamide stored at 4°C
- ◆ Dextran sulfate at 50% in distilled water, stored at 4°C
- ◆ 20 x standard saline citrate (SSC): 3 M NaCl, 0.3 M sodium citrate, pH 7, stored at 4°C
- ◆ Ice

Method^a

- 1 Prepare 25 µl of hybridization solution: 2 to 10 µg/ml biotin or digoxigenin labelled double-stranded DNA^b in 50% formamide, 2 x SSC, 10% dextran sulfate and 400 µg/ml competitor tRNA or salmon sperm DNA or *Escherichia coli* DNA.^c
- 2 Store the hybridization solution at 4°C until use.
- 3 Denature the hybridization solution just before use as follows^d.
- 4 Plunge the plastic container (generally a microcentrifuge tube) containing the hybridization solution into boiling water for 4 min.
- 5 Then, immediately chill the container in melting ice to avoid reassociation of the heat denatured molecules.
- 6 Use cold hybridization solution for hybridization experiments within 10 min after the end of the heat treatment (see [Post-embedding in situ hybridization and detection of hybrids](#)).
- 7 Store the remaining solution at 4 °C where it will remain useful for up to three years.
- 8 Heat treat the solution before each subsequent use.

Notes

- a We have chosen to keep constant the composition of the hybridization solution and to adjust stringency by varying the hybridization temperature.
- b Oligonucleotide probes can be used instead of double-stranded DNA probes.

- c *Escherichia coli* DNA is used as a competitor for ribosomal DNA probes. Salmon sperm DNA, which displays some sequence homology with portions of the ribosomal genes, is employed with the other DNA probes. tRNA is employed preferentially with oligonucleotide probes because it eliminates the necessity of a heat treatment of the hybridization solution prior to the hybridization step.
- d Hybridization solution containing double-stranded DNA probes must be submitted to a heat treatment just before use in order to denature the double-stranded molecules and to allow subsequent base pairing of the resulting single-stranded probes with the complementary sequences of the biological material.