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Calf Thymus DNA Solution

Cat. No. 15633-019 **Size: 5 × 1 ml**
Conc.: Approximately 10 mg/ml **Store at -20°C.**

Description

Calf Thymus DNA Solution was developed for use in hybridization protocols as a blocking agent to reduce the non-specific binding of a hybridization probe to the surface of the filter. It is not designed to serve as a DNA standard or reference solution. Calf Thymus DNA Solution is prepared from highly pure, phenol-chloroform extracted DNA, and DNase-free, RNase-free (DEPC-treated), distilled, deionized water. Once dissolved, the DNA solution is sheared to an average size of ≤ 2000 bp. Carrier DNA is typically used at a concentration of 100 µg/ml in both the prehybridization and hybridization solutions.

Quality Control

Concentration: Approximately 10 mg/ml (A_{260}) Concentration may vary from 9.5 to 11 mg/ml.

Size Range: Majority of DNA ≤ 2000 bp (1% TAE agarose gel)

Instructions For Use

Use Calf Thymus DNA Solution directly in the preparation of both prehybridization and hybridization solutions for nucleic acid hybridization procedures. Although the exact formulae for these solutions may vary somewhat in composition, a typical solution may contain 6X SSC [0.9 M sodium chloride, 0.09 M sodium citrate, (pH 7.0)], 5X Denhardt's solution [0.1% (w/v) polyvinylpyrrolidone, 0.1% (w/v) Ficoll type 400, 0.1% (w/v) bovine serum albumin] and 100 µg/ml sheared, denatured DNA. To reduce the temperature at which the hybridization step is performed, formamide is added to these solutions.

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Examples of both formamide and non-formamide prehybridization and hybridization solutions are provided below and on the next page.

Formamide Formulation

Stock Solution	Prehybridization Volume (ml)	Hybridization Volume (ml)
20X SSC	3.0	3.0
50X Denhardt's Solution	1.0	1.0
Calf Thymus DNA (10 mg/ml)	0.1	0.1
2 M Sodium phosphate, pH 6.5	0.125	0.1
Formamide	5.0	--
10% Dextran sulfate* (in formamide)	--	5.0
H_2O	0.775	0.8
Total Volume	10.0	10.0

*To prepare 10% dextran sulfate, dissolve 5 g dextran sulfate in formamide to a final volume of 50 ml. High-molecular weight dextran sulfate dissolves slowly. Stir the solution slowly overnight at room temperature and store at -20°C.

Prehybridization and hybridization procedures performed using formamide-containing solutions are generally used at 42°C. However, hybridization parameters should be optimized for the particular system and the probe being used.

Non-Formamide Formulation

Stock Solution	Prehybridization Volume (ml)	Hybridization Volume (ml)
20X SSC	3.0	3.0
50X Denhardt's Solution	1.0	1.0
Calf Thymus DNA (10 mg/ml)	0.1	0.1
10% SDS	0.5	0.5
0.5 M EDTA, pH 8.0	--	0.2
H_2O	5.4	5.2
Total Volume	10.0	10.0

Prehybridization and hybridization procedures using non-formamide containing solutions are generally used in hybridizations at 62-68°C. However, when oligomers are used as probes the hybridizations are performed at 5-10°C below the calculated T_m of the oligomers.

Procedure

1. Place the Southern filter in a hybridization bag.
2. Add prehybridization solution (0.2 ml/cm^2 of filter).
3. Remove all the air bubbles and seal the bag.
4. Submerge the bag in a water bath at the appropriate temperature (42°C for formamide or 65°C for non-formamide), for 1-2 hours.
5. Add freshly denatured, labeled probe to the hybridization solution.
6. Cut open one side of the hybridization bag and remove all the prehybridization solution.
7. Add the hybridization solution (0.05 ml/cm^2 of filter), containing the probe to the hybridization bag.
8. Repeat step 3 and incubate at the appropriate temperature for the required time [Refer to Maniatis (1) or Meinkoth (2)].

Note

If only a small amount of prehybridization or hybridization solution is to be used each time, aliquot the stock and store at -20°C.

Reference

1. Maniatis, T., *et al. Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, 2nd ed., 1989.
2. Meinkoth, J. and Wahl, G. (1984) *Analytical Biochemistry 138*:267.

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