Herring Sperm DNA Solution

Cat. No. 15634-017 **Size: 5 × 1 ml**
Conc.: 10 mg/ml **Store at -20°C.**

Description:
Herring Sperm 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 and the concentration is adjusted to 10 mg/ml. Herring Sperm 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. Carrier DNA is typically used at a concentration of 100 µg/ml in both the prehybridization and hybridization solutions.

Quality Control:
Concentration: 10 mg/ml (A_{260})
Size Range: Majority of DNA ≤ 2000 bp (1% TAE agarose gel)

Instructions For Use:
Herring Sperm DNA Solution can be used directly in the preparation of both prehybridization and hybridization solutions used in 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 can also be added to these solutions.

Examples of both formamide and non-formamide prehybridization and hybridization formulations are given on the following pages.

Doc. Rev06/11/01

FORMAMIDE FORMULATION:

Stock Solution	Prehybridization Volume (ml)	Hybridization Volume (ml)
20X SSC	3.0	3.0
50X Denhardt's Solution	1.0	1.0
Herring Sperm 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
Herring Sperm 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.
9. Repeat step 3 and incubate at the appropriate temperature for the required time [Refer 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.