

Preparation of BLOTTO-10 Solution (for Southern Blotting)

Adopted from BV's Cookbook by T.-C. He 10/11/2002

For Making Four Liters

Add:

40 g	SDS (or use 10% SDS stock solution)
20 g	milk powder (carnation fat free milk, buy it in grocery store)
240 g	PEG (Sigma P2139, i.e., Ave. MW >8kd)
1000ml	20xSSPE (from AMRESCO- 0810-4l or Life Tech, or homemade)

Mix well, then add:

400 ml	Formamide (EM-molecular biology grade or Sigma or Fisher)
40 ml	sssssDNA (2 mg/ml stock prepared according to lab recipe)
~2360 ml	deionized distilled water (final vol. = 4000ml)

Dissolve by heating/incubating in 60⁰C water bath for a few hours until solution is clear.

Mix with magnetic stirrer at RT for an additional hour.

Aliquot into 100-500ml bottles

store at -20⁰C or -80⁰C.

When ready to use, melt at 65⁰C and mix well (don't boil---If DNA precipitates, it will NOT work).

APPENDIX:

20x SSPE (for 1000ml)

NaCl

175.3g

NaH ₂ PO ₄ ·H ₂ O	27.6g
EDTA, 0.5M	40ml

Adjust pH to 7.4 (use diluted NaOH)

Add ddH₂O to 1000ml