

Recipes for reagents used for In Situ Hybridization

Stock Proteinase K (10mg/ml)

Sigma P-0390 Type XI

Sigma Chemical
P.O. Box 14508
St Louis MO 63178-9916
1-800-325-3010

100 mg - add 10 ml DEPC treated water
Aliquot 120 μ l
Store at -70°C

use 1-2 μ g/ml

RNase A (10 mg/ml)

Sigma R-5125 type IIIA

Add 10 ml of RNase buffer to 100mg
Aliquot 0.4 ml
Store at -70°C

1 M DTT for prehyb mix

Sigma D 9779

1 g in 6.5 ml DEPC H₂O or
5 g in 26 ml DEPC H₂O

Aliquot 1 ml per tube
Store at -70°C

Dextran Sulfate for Prehyb mix

25 g dextran sulfate in 50 ml tube
Bring to 50 ml with DEPC H₂O

As dextran sulfate goes into solution, warm to 65°C to get into solution or let sit overnight at room temp

Aliquot 6 ml in 15 ml tubes
Store at -70°C

Warm to 65°C when ready to use

50x Denhardt's Reagent

**THIS CAN BE PURCHASED
FROM SIGMA
#D 2532**

Treat all containers with DEPC water

5 g Ficoll (Type 400, Pharmacia)
5 g polyvinyl pyrrolidone
5 g BSA (pentex fraction V)
500 ml DEPC H₂O
Filter

5 M NaCl

CAN PURCHASE FROM GIBCO

1000 ml
292 g NaCl
700 ml dH₂O

2000 ml
584 g NaCl
1500 ml dH₂O

3000 ml
876 g NaCl
2000 ml dH₂O

Warm to 65°C to dissolve

QS to final volume

Add 500 ul DEPC/ liter, mix well and let sit overnight

Autoclave

1 M Tris, pH 8

CAN PURCHASE FROM GIBCO

121.1 g Trizma Base (Sigma T-1503)

1000 ml DEPC H₂O

pH to 8.0

****Do not add DEPC to solution - USE DEPC WATER - DON'T AUTOCLAVE*****

Prehyb Mix for Riboprobes

<u>FINAL CONCENTRATION</u>	<u>Stock</u>	<u>30 ml</u>	<u>90 ml</u>
50% formamide (deionized)	100%	15.0 ml	45 ml
0.3 M NaCl	5M	1.8 ml	5.4 ml
20 mM Tris	1 M	600 ul	1.8 ml
5mM EDTA	250mM	600 ul	1.8 ml
Denhardts 1x	50X	600 ul	1.8 ml
10% dextran sulfate	50%	6.0 ml	18 ml
10 mM DTT	1100 mM	300 ul	900 ul
500 ug/ml yeast tRNA	25 mg/ml	600 ul	1.8 ml
sterile ddH2O (nuclease free)		4.5 ml	13.5 ml

Aliquot 5 ml and store at -70°C

20X SSC

1000 ml
175.3 g NaCl
88.2 g sodium citrate
800 ml dH2O

PURCHASE FROM GIBCO

4000 ml
701.2 g NaCl
352.8 g sodium citrate
3200 ml dH2O

Adjust pH to 7.0 then QS to final volume

For DEPC solution - add DEPC to final concentration of 0.1% (1 ml/ liter) , let sit overnight and autoclave. Or use ddH2O to make up solutions.

	<u>1000 ml</u>	<u>4000 ml</u>
<u>0.5 x SSC</u>		
20x SSC	25 ml	100 ml
dH2O	975 ml	3900 ml
<u>0.1 X SSC</u>		
20x SSC	5 ml	20 ml
dH2O	995 ml	3980 ml
<u>2 X SSC</u>		
20x SSC	100 ml	400 ml

dH2O	900 ml	3600 ml
------	--------	---------

0.1SSC / 0.5% Tween

20x SSC	5 ml	20 ml
dH2O	995 ml	3960 ml
Tween 20	5 ml	20 ml

Proteinase K / RNASE Buffer

	<u>Stock</u>	<u>1000 ml</u>	<u>4000 ml</u>
500 mM NaCl	5 M	100 ml	400 ml
10mM Tris pH 8.0	1 M	10 ml	40 ml
dH2O		890 ml	3560 ml

For prehyb PK buffer - use DEPC or ddH2O treated water to make solution. For posthyb RNase buffer, use regular dH2O

Box Buffer (for humidity box)

	<u>Stock</u>	<u>200 ml</u>	<u>500 ml</u>
50% formamide (cheap stuff)	100%	100 ml	250 ml
0.3M NaCl	5M	12 ml	30 ml
10 mM Tris pH 8.0	1M	2 ml	5 ml
1 mM EDTA	0.2M	1 ml	2.5 ml
ddH2O	-	85 ml	212.5 ml