

1 x PBS pH 7.5

- Dissolve 10 Tablets into 1 litre of ddH₂O
- DEPC treat - add 1 ml of DEPC to 1 litre of PBS, let it sit overnight in a fume hood and Autoclave

PBSAT (1 x PBS + 0.1% Tween-20)

- Dissolve 10 Tablets into 800 ml of ddH₂O
- Adjust to 1 litre with ddH₂O
- Add 1 ml of DEPC into solution
- Shake well and leave overnight at 37°C with the bottle cap-loose
- Sterilise by autoclaving
- When cool add 1 ml of Tween 20
- Filter sterilise and store @ RT

75% EtOH in PBSAT

- 375 ml EtOH + 125 ml PBSAT
- Store @ RT

50% EtOH in PBSAT

- 250 ml EtOH + 250 ml PBSAT
- Store @ RT

Proteinase K @ 25 mg/ml

- Dissolve 0.125 g of Proteinase K in 5 ml of Sterile DEPC H₂O
- Aliquot into 1 ml Eppendorfs and store @ -20°C

0.1 M Triethanolamine (TEA), pH 7 – 8

- Stock Triethanolamine (T-1377; Sigma) – **Concentration 7.53M**
- Prepare a 1/75.3 dilution of the stock TEA:

6.65 ml 7.53 TEA stock
493.35 ml autoclaved DEPC treated H ₂ O

- Note: use pH strips to adjust pH with HCL
- Filter-sterilise, and keep @ RT

4% (w/v) Paraformaldehyde in PBSAT

- Dissolve 8g Paraformaldehyde in 200 ml PBSAT
- Filter-sterilise (0.2 µ filter units)
- Store @ - 20°C in 50 ml aliquots

10% CHAPS (w/v solution)

- Dissolve 5 g of CHAPS in 40 ml of sterile DEPC ddH₂O.
- Adjust volume to 50 ml with sterile DEPC ddH₂O
- Store in aliquots @ -20C.

10% Tween 20 (v/v solution)

- 10 ml of Tween 20
- 90 ml of sterile DEPC treated ddH₂O

Heparin (100mg/ml) Stock

- Dissolve 0.5 g of Heparin in 5 ml of sterile water (**not** DEPC water)
- Store in 0.15 ml aliquots @ -20°C.

Yeast RNA (100 mg/ml) Stock

- Store in 0.15 ml aliquots @ -20°C

0.5M EDTA, pH8.0

- From SIGMA, cat# E7899; DNase, RNase free

8 M Lithium Chloride

- From SIGMA, cat# L7026; DNase, RNase free

20 x SSC (3M NaCl, 0.3 M tri-sodium citrate), pH 7

175.3 g NaCl
88.2 g Tri-sodium citrate
DEPC ddH ₂ O to ~800 ml

- Dissolve solutes and adjust pH to 7.0 with HCl
- Adjust volume to 1 litre with DEPC-ddH₂O.
- Sterilise by autoclaving

Hybridisation Buffer

<i>Final Concentration</i>	<i>Storage</i>	<i>Stock</i>	<i>100 ml</i>
50% Formamide (deionized)	4°C if new, or -20°C aliquots	100%	50.0 ml
5 x SSC (pH 7)	Bench	20 x SSC	25.0 ml
1 mg/ml total yeast RNA	-20°C	100 mg/ml	1.0 ml
100 µg/ml Heparin	-20°C	100 mg/ml	0.1 ml
1 x Denhardt's	-20°C	50 x Denhardt's	2.0 ml
0.1% Tween 20	Bench	10% Tween 20	1.0 ml
0.1% CHAPS	Bench	10% CHAPS	1.0 ml
10 mM EDTA pH 8.0		0.5M EDTA pH 8.0	2.0 ml
DEPC water	Bench		17.9 ml

Note:

- Make up Hybridisation buffer without the yeast RNA (Hybridisation [-] buffer)
- Aliquot 9.9 ml of the buffer into sterile 15 ml Falcon tubes and freeze @ -20°C
- To use thaw an aliquot of the hybridisation buffer and add 100 µl of 100 mg/ml total yeast RNA to make Hybridisation [+] buffer

Formamide and Hybridisation

- Formamide lowers the melting point of nucleic acids so that the strands separate more readily.

- DNA is normally more stable in a double-stranded structure (even if every base isn't complementary) and less stable when single-stranded, so formamide must increase the stability of single-strandedness.
- In situ hybridization, an RNA probe binds to mRNA that is already single-stranded.
- mRNA does not gain any stability by being a hybrid unless the probe is specific and can bind properly, thus increasing stability. For example, in the presence of formamide, a U nucleotide would rather bind to an A than nothing (binding to specific probe is better than staying single stranded), but a U nucleotide would rather bind to nothing than a G (binding to non specific probe is worse than binding to nothing)

Denhardt's solution (for use in RNA work)

- A solution commonly used during probe hybridisation, Denhardt's solution is a mixture of high-molecular weight polymers capable of saturating non-specific binding sites and artificially increasing the concentration of available probe.
- It is prepared as a 50 x solution with the following composition:

1%	Ficoll (type 400)
1%	polyvinylpyrrolidone
1%	bovine serum albumen
- Dissolve components in DEPC treated or sterile ddH₂O

Tween 20

- Tween 20 is a polysorbate surfactant whose stability and relative non-toxicity allows it to be used as a detergent and emulsifier or as a blocking agent
- Tween 20 is also needed in the buffer to further prevent the non-specific binding

2 x SSC + 0.1 % Tween 20

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|--------|----------------------------|
| 100 ml | 20 x SSC |
| 890 ml | sterile ddH ₂ O |
| 10 ml | 10% Tween 20 |
- Filter-sterilise and store @ RT

0.2 x SSC + 0.1 % Tween 20

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|--------|----------------------------|
| 10 ml | 20 x SSC |
| 980 ml | sterile ddH ₂ O |
| 10 ml | 10% Tween 20 |
- Filter-sterilise and store @ RT

1M MAB (Maleic Acid Buffer), pH 7.8 - 1 litre

- Dissolve 116.1 g Maleic acid in a small amount of DEPC dd H₂O (300 ml) pH to 7.8 with lots of 10N NaOH
- Sterilise by autoclaving

MAB (Maleic Acid Buffer), pH 7.8 - 1 litre

<i>1. MAB made from stock solutions:</i>	Final Concentration
100 ml 1M Maleic acid	0.1 M
30 ml 5M NaCl	0.15 M
10 ml 10% Tween 20	0.1% (v/v Tween 20)
860 ml Sterile DEPC ddH ₂ O	
<i>2. MAB made from stock chemicals:</i>	Final Concentration
100 mM Maleic acid	23.21 g
150 mM NaCl	17.53 g
0.1% Tween 20	20 ml of 10% Tween 20 stock

- Dissolve in approximately 1800 ml ddH₂O, adjust pH to 7.5 with 10 N NaOH, volume to 2 litres
- Store @ RT

MAB + 2% (w/v) Blocking Reagent

- 10 g Blocking Reagent in 500 ml MAB
- Autoclave and store @ 4°C

Lamb Serum

- Lamb serum purchased from Gibco BRL (cat # 16070-096) is thawed @ RT
- Heat-inactivate complement @ 60°C for 30 min to destroy endogenous alkaline phosphatase activity
- Centrifuge @ 10,000 rpm for 20 min @ 4°C to remove particulate material
- Store @ -20°C in 25 ml aliquots

MAB + 2% (w/v) Blocking Reagent + 20% (v/v) Lamb serum

- 20 ml of heat-inactivated Lamb serum + 80 ml MAB with 2% Blocking Reagent
- NB: Make fresh as needed, and do not store more than 1 – 2 days @ 4°C

Anti-Digoxigenin AP, Fab (Antibody)

- From Roche, cat # 11 093 274 910 (150 U in 200 µl)

1M Tris-HCl, pH 9.5

- Dissolve 121.14 g Tris (hydroxymethyl) aminomethane, (Tris, MW = 121.14) in 800 ml ddH₂O
- Adjust pH to desired value by adding concentrated HCl (pH 9.5 : ~ 8 ml)
- Adjust volume to 1 litre with ddH₂O
- Sterilise by autoclaving and store @ RT
- Do not treat Tris solutions with DEPC

1 M MgCl₂ - 1 litre

- Dissolve 203.31 g magnesium chloride-6 H₂O, (MW=203.31) in 800 ml ddH₂O
- Adjust volume to 1 litre with ddH₂O
- Sterilise by autoclaving and store @ RT

5M NaCl - 1 litre

- Dissolve 292.2 g sodium chloride, (MW=58.44) in 800 ml dd H₂O
- Adjust volume to 1 litre with ddH₂O
- Sterilise by autoclaving and store @ RT

Alkaline Phosphatase Buffer

- Store @ 4°C

<i>Final Concentration</i>	<i>50 ml</i>	<i>100 ml</i>
100 mM Tris Cl, pH 9.5	5.0 ml 1 M Tris Cl, pH 9.5	10.0 ml of the same
50 mM MgCl ₂	2.5 ml 1 M MgCl ₂	5.0 ml of the same
100 mM NaCl	1.0 ml 5M NaCl	2.0 ml of the same
0.1% Tween 20	0.5 ml 10% Tween 20	1.0 ml of the same
Sterile ddH ₂ O	41.0 ml	82.0 ml

NBT/BCIP Stock Solution

- From Roche, 11 681 451 001
- Solution of 18.75 mg/ml nitroblue tetrazolium chloride and 9.4 mg/ml 5-bromo-4-chloro-3-indolylphosphate, toluidine salt in 67% (DMSO) (v/v)

50% EtOH/PBS

- 125 ml EtOH + 125 ml PBS
- Store @ RT

70% EtOH/PBS

- 175 ml EtOH + 75 ml PBS
- Store @ RT

90% EtOH/PBS

- 225 ml EtOH + 25 ml PBS
- Store @ RT

*100% EtOH**BA:BB clearing agent (1:2 Benzyl Alcohol/Benzyl Benzoate)*

- 100 ml Benzyl alcohol
- 200 ml Benzyl Benzoate
- Store @ RT