

II. Reagents and Recipes: Molecular Biology

In situ hybridization <[back to insitu](#)>:

Hybridization Buffer - Store at -20°C

Components	For 100ml	400 ml
50% deionized formamide **	50.0 ml Formamide	200ml formamide
5X SSC	25.0ml 20X SSC	100ml 20XSSC
100ug/ml Heparin	10mg of heparin	40mg Heparin
0.1% Tween-20	1.0ml of 10% w/v Tween 20	4.0 ml 10% Tween
1mg/ml tRNA	2.0ml of 50mg/ml yeast tRNA	400 mg tRNA dissolved in 8ml DEPC-ddH2O
1X Denhardts	2.0ml of 50X Denhardts	8ml of 50X Denhardts
0.1% CHAPS	1.0ml of 10% w/v CHAPS	4 ml 10% CHAPS
5mM EDTA, pH 7.2 (8 is OK)	1.0ml 0.5M EDTA.	4ml 0.5M EDTA,
DEPC-ddH2O	Bring to 100ml	Bring to 400ml

** formamide used for pre-hyb solution needs to be deionized and kept frozen when not used. This will prevent unnecessary hydrolysis of probe.

Blocking Buffer Stock (5X; Roche) for post-hybridization– make 10% stock following product spec sheet.

- 50g dry powder into 500ml Maleate Buffer. Heat to around 60C to dissolve.
- Store aliquots at -20C.

Blocking Buffer (1X) for post hybridization – “working conc.”

- Dilute stock blocking agent to 2% in Maleic acid buffer.

Buffer 1 (for 1 Liter)

(150mM NaCl, 100mM Tris; pH 7.5)

1. add **30ml** of **5M NaCl**
2. add **100ml** of **1M Tris, pH7.5**
3. bring volume to 1 liter with ddH2O.
4. filter through 0.45 µm membrane

Buffer 3 (for 1 Liter) *check pH

(100mM NaCl, 100mM Tris pH9.5, 50mM MgCl₂)

1. add **20 ml** of **5M NaCl**
2. add **100ml** of **1M Tris, pH 9.5**
3. add **50ml** of **1M MgCl₂**
4. bring volume to 1 liter with ddH2O.
5. filter with 0.45 membrane filter

EDTA (0.5M, pH8.0) - for 500ml

1. add 93.05g EDTA (disodium ethylenediaminetetraacetate-2H₂O to 400ml of ddH₂O.
2. **Add 0.5ml of DEPC**
3. adjust volume to 500ml with ddH₂O.
4. Mark level and autoclave.
5. Replace lost volume with DEPC-H₂O.
6. Dispense into 50ml aliquots.
*note-EDTA will not go into solution until the pH is adjusted to 8.0.

Formamide (50%) with 2X SSC (400ml)

1. 200ml formamide
2. 40ml 20XSSC
3. add 160ml ddH₂O

Formamide (50%)with 2X SSC & 0.1% CHAPS (400ml)

1. 200ml formamide
2. 40ml 20XSSC
3. 156 ml ddH₂O
4. 4ml 10% CHAPS

Magnesium Chloride (1M); FW=203.3

1. dissolve 101.65g of MgCl₂·6H₂O into 400ml of ddH₂O.
2. Add 0.5ml of DEPC and adjust volume to 500ml with ddH₂O Mark bottle.
3. Sterilize and remove DEPC by autoclaving.
4. Adjust volume to 500ml with DEPC-H₂O and dispense into 50ml tubes.

Maleate buffer (for 1 Liter)

(100mM maleic acid, pH 7.5, 150mM NaCl)

1. add 11.6g Maleic acid
2. add 8.76g NaCl
3. add 7.0g NaOH pellet to 800ml ddH₂O.
4. Adjust pH to 7.5.
5. sterilize by autoclaving.

NaCl (5M); FW=58.44

1. Dissolve 146g of NaCl into 400ml of

- ddH₂O.
2. Add 0.5ml of DEPC and adjust volume to 500ml with ddH₂O Mark bottle.
 3. Sterilize and remove DEPC by autoclaving.
 4. Adjust volume to 500ml with DEPC-H₂O and dispense into 50ml tubes.

Proteinase K

- Dissolve 100mg into 10ml of (10mM Tris HCl, pH7.5, 20mM CaCl, and 50% glycerol) to give a 1000X stock (10mg/ml) Prot. K soln.
- Alternatively; dissolve in water

SSC (0.2X) with 0.1% Tween

1. 10ml 20XSSC
2. 1ml Tween
3. bring volume to 1 Liter with ddH₂O

TE Buffer (10x stock) – 500ml

1. add 5 ml of 1 M Tris-HCl (pH 8.0)
2. add 1ml of 0.5 M EDTA
3. bring volume to 500ml of ddH₂O

Media Preparation for bacteria:

Antibiotics

antibiotic	[stock] mg/ml	[workin g] µg/ml	diluent
Ampicillin*	4	50	ddH ₂ O
Carbenecillin	4	50	50%EtOH/50% ddH ₂ O
Kanamycin	10	30	ddH ₂ O
Streptomycin	50	30	ddH ₂ O
Tetracycline	12	12	ddH ₂ O

* carbenicillin, at the same concentration can be substituted for ampicillin.

Note- all antibiotics should be dissolved in sterile ddH₂O unless otherwise noted. Store at -20°C.

Glycerol stock solution for bacterial storage (65% glycerol (vol/vol), 0.1M MgSO₄, 0.025 M Tris-HCl, pH 8.0; *as per Current Protocols*) add the following:

- 32.5ml glycerol
 - 5 ml of 1M MgSO₄
 - 1.25 ml 1M Tris-HCl, pH 8.0
 - adjust volume to 50ml w/ sterile H₂O
- ** you will add 2 ml's of mid-log culture or 1 ml of freshly saturated culture to 1 ml

glycerol solution to make a glycerol stock. Store this @ -80C.

GYT medium, per liter

- 10% (v/v) glycerol
- 0.125% (w/v) yeast extract
- 0.25% (w/v) tryptone
- sterilize the medium by passing it through a pre-rinsed 0.22µm filter. Store in 2.5ml aliquots at 4°C.

LB medium (*Luria-Bertani*), per liter “Current protocols”

- 10g tryptone
- 5g yeast extract
- 5g NaCl
- 1ml 1N NaOH

LB medium (*Luria-Bertani*), per liter “Qiagen recipe”

- 10g tryptone
- 5g yeast extract
- 10g NaCl
- 1ml 1N NaOH

LB agar plates, per liter

- 10g tryptone
- 5g yeast extract
- 5g NaCl
- 1ml 1N NaOH
- 15g agar or agarose
autoclave or microwave (for 10 minutes at full boil to kill bacteria).
Additives: if required add ampicillin (carbenecillin works the same) at 50µg/ml, tetracycline at 12 µg/ml or Kanamycin at 40µg/ml.

Magnesium sulfate (1M MgSO₄), per 250ml

- 61.63 g MgSO₄ (FW-246.5)
- add 250ml ddH₂O
- autoclave and store at RT.

SOB medium without Mg⁺⁺, per liter

- To 950 ml of ddH₂O, add:
- 20g Tryptone
- 5 g Yeast Extract
- 0.5 g NaCl
- shake until dissolved. Add 10 ml of