Appendix C

Buffers and Stock Solutions

Alkaline Lysis Solution I
50 mM glucose
25 mM Tris-Cl (pH 8.0)
10 mM EDTA (pH 8.0)
Autoclaved and stored at 4°C.

Alkaline Lysis Solution II
0.2 N NaOH (freshly diluted from a 10 N stock)
1% (w/v) SDS
Prepare Solution II fresh and use at room temperature.

Alkaline Lysis Solution III
5 M potassium acetate, 60.0 ml
glacial acetic acid, 11.5 ml
H₂O, 28.5 ml
The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. Store the solution at 4°C and transfer it to an ice bucket just before use.

A. platensis growth medium:
It is BG11 + ASNIII (1:1, v/v) – 1000 ml medium which had the following component.

Medium BG11 - 500 ml
Medium ASNIII - 500 ml
A5 + Co trace metal mix – 1 ml

BG-11 medium had the following composition (per liter):
1.5g NaNO₃ (17.65mM)
0.04g K₂HPO₄.3H₂O (0.18mM)
0.075g MgSO₄.7H₂O (0.30mM)
0.036g CaCl₂.2H₂O (0.25 mM)
0.006g citric acid (0.03mM)
0.006g ferric ammonium citrate (0.03mM),
0.001g EDTA (0.003mM disodium salt),
0.04g Na₂CO₃ (0.38 mM).

**ASN -III media had the following composition (per liter):**

25g NaCl (427 mM),
2.0g MgCl₂.6H₂O (9.8mM),
0.5g KCl (6.7mM),
0.75g NaNO₃ (8.8mM),
0.02g K₂HPO₄.3 H₂O (0.09mM),
3.5g MgSO₄.7 H₂O (14.2mM),
0.5g CaCl₂.2H₂O (3.4mM),
0.003g citric acid (0.015mM),
0.003g ferric ammonium citrate (0.015mM),
0.0005g EDTA (0.0015mM disodium salt),
0.04g Na₂CO₃ (0.38 mM).

**A5 + Co trace metal mix had the following composition (per liter):**

2.86g H₃BO₃
1.81g MnCl₂
0.222g, ZnSO₄
0.390g Na₂MoO₄
0.079g CuSO₄.5 H₂O
0.0494g Co (NO₃)₂.6 H₂O.

Each component of A5 + Co trace mix was dissolved in a small volume of sterile water, the solutions were then mixed in the order indicated above and stored at 4°C for 6 months.

The media was autoclaved at 121 °C for 20 minutes. In all cases, the media were sterilized by autoclaving without citric acid, K₂HPO₄.3 H₂O, Na₂CO₃ and ferric ammonium citrate & EDTA which were filter sterilized and stored individually at 4°C and added just before mixing of the BG11 and ASN 111 media.
EDTA
To prepare EDTA at 0.5 M (pH 8.0): Add 186.1 g of disodium EDTA•2H₂O to 800 ml of H₂O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (approx. 20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approx. 8.0 by the addition of NaOH.

Glycerol
To prepare a 10% (v/v) solution: Dilute 1 volume of molecular-biology grade glycerol in 9 volumes of sterile pure H₂O. Sterilize the solution by passing it through a pre-rinsed 0.22-μm filter. Store in 200-ml aliquots at 4°C.

LB Broth with Supplements
Prepare 1 liter of LB broth, Autoclave and Add the following filter-sterilized supplements prior to use 10 ml of 1 M MgSO₄, 10 ml of 20% (w/v) maltose.

NZY Broth (per Liter)
5 g of NaCl
2 g of MgSO₄•7H₂O
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Add deionized H₂O to a final volume of 1 liter adjusted the pH to 7.5 with NaOH and then autoclave

NZY Agar (per Liter)
Add 15 g of agar to NZY broth and adjust the pH to 7.5 with NaOH. Autoclave and pour into petri dishes.

NZY Top Agar (per Liter)
Add 0.7% (w/v) agarose to 1 liter of NZY broth and autoclave.
SM Buffer (per Liter)
5.8 g of NaCl
2.0 g of MgSO₄ · 7H₂O
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add deionized H₂O to a final volume of 1 liter.

SDS
To prepare a 20% (w/v) solution, dissolve 200 g of electrophoresis-grade SDS in 900 ml of H₂O. Heat to 68°C and stir with a magnetic stirrer to assist dissolution. If necessary, adjust the pH to 7.2 by adding a few drops of concentrated HCl. Adjust the volume to 1 liter with H₂O. Store at room temperature.

TE
100 mM Tris-Cl (desired pH)
10 mM EDTA (pH 8.0)
(10x Tris EDTA) Sterilize solutions by autoclaving and store the buffer at room temperature.

Sodium Acetate (3M)
Dissolve 408.3 g of sodium acetate.3H₂O in 800 ml of H₂O. Adjust the pH to 5.2 with glacial acetic acid or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 1 liter with H₂O. Dispense into aliquots and sterilize by autoclaving.

Bromophenol Blue Solution (0.4%, w/v)
Dissolve 4 mg of solid bromophenol blue in 1 ml of sterile H₂O. Store the solution at room temperature.

Ethidium Bromide
Add 1 g of ethidium bromide to 100 ml of H₂O. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer the 10 mg/ml solution to a dark bottle and store at room temperature.
Ammonium Acetate
To prepare a 10 M solution in 1 liter, dissolve 770 g of ammonium acetate in 800 ml of H2O. Adjust volume to 1 liter with H2O. Sterilize by filtration.

IPTG
IPTG is isopropylthio-β-D-galactoside. Make a 20% (w/v, 0.8 M) solution of IPTG by dissolving 2 g of IPTG in 8 ml of distilled H2O. Adjust the volume of the solution to 10 ml with H2O and sterilize by passing it through a 0.22-μm disposable filter. Dispense the solution into 1-ml aliquots and store them at -20°C.

X-gal Solution
X-gal is 5-bromo-4-chloro-3-indolyl-β-D-galactoside. Make a 2% (w/v) stock solution by dissolving X-gal in dimethylformamide at a concentration of 20 mg/ml solution. Use a glass or polypropylene tube. Wrap the tube containing the solution in aluminum foil to prevent damage by light and store at -20°C.

SSC (20 X)
Dissolve 175.3 g of NaCl and 88.2 g of sodium citrate in 800 ml of H2O. Adjust the pH to 7.0 with a few drops of a 14 N solution of HCl. Adjust the volume to 1 liter with H2O. Dispense into aliquots. Sterilize by autoclaving.

Wash Solution 1
2x SSC
0.1% (w/v) SDS

Wash Solution 2
1x SSC
0.1% (w/v) SDS

Wash Solution 3
0.1x SSC
0.1% (w/v) SDS

Gelatin
To prepare a 2% (w/v) solution: Add 2 g of gelatin to a total volume of 100 ml of H₂O and autoclave the solution.

**MgSO₄**
To prepare a 1 M solution: Dissolve 12 g of MgSO₄ in a final volume of 100 ml of H₂O. Sterilize by autoclaving or filter sterilization. Store at room temperature.

**6x Gel-loading Buffer**
0.25% (w/v) bromophenol blue
0.25% (w/v) xylene cyanol FF
30% (v/v) glycerol in H₂O

**Nitrite Estimation Catalyst Solution**
Solution A: 31.36 mg CuSO₄ in 100 ml
Solution B: 10 mg ZnSO₄ in 10 ml CuSO₄ solution and make up volume to 100 ml

**Hydrazine sulphate stock solution**
Dissolve 152 mg in 100 ml double distilled water.

**RNA isolation extraction buffer**
100 mM Lithium chloride
100 mM Tris (pH: 8.0)
10 mM EDTA
1 % SDS

**RNA loading buffer (1 ml)**
1 mM EDTA
50 % Glycerol
0.4 % Bromophenol Blue
DEPC treated water