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APPENDIX A

MEDIA AND SOLUTIONS

All media were autoclaved at 121 °C for 20 min prior to use, unless otherwise specified.

Water used for making solutions, media and diluting buffers was purified using a Milli-RO Plus (Millipore) water purification system. Ultrapure water used was obtained by further purification of the above water using a Milli-Q Plus (Millipore) water purification system.

A.1 MEDIA

A.1.1 Artificial Sea Water (ASW)

NaCl (Saarchem)	24.7 g
$MgCl_2.6H_2O$ (Saarchem)	4.7 g
KCl (Saarchem)	0.66 g
$CaCl_2.2H_2O$	1.9 g
$MgSO_4.7H_2O$ (Saarchem)	6.3 g
$NaHCO_3$	0.18 g
Water to	1 L

Autoclave

A.1.2 Fe-solution

$Fe(NH_4)_2(SO_4)_2.6H_2O$	702 mg
Na_2 EDTA	600 mg
Water to	1 L

A.1.3 PII metal solution

Na_2 EDTA	100 mg
H_3BO_3	114 mg
$FeCl_3.6H_2O$	4.9 mg
$MnSO_4$	16.4 mg
$ZnSO_4.7H_2O$	2.2 mg
$CoSO_4.7H_2O$	0.48 mg

Water to	100 ml

A.1.4 PES-enriched seawater medium (1/3 strength) (Provasoli, 1968)

$NaNO_3$	350 mg
Na_2 glycerophosphate $5H_2O$	50 mg
Fe solution	25 ml
PII	25 ml
Vitamin B_{12}	10 µg
Thiamine	0.5 mg
Biotin	5 µg
Tris buffer (Sigma Co.)	500 mg
Water to	100 ml

Adjust pH to 7.8, autoclave and store at 10 °C.
Add 6.6 ml to 1 L ASW.

A.1.5 PES-N (ES medium lacking nitrogen)

Prepared exactly the same as ES medium except no $NaNO_3$ is added and $Fe_2(SO_4)$ instead of $Fe(NH_4)_2(SO_4)_2.6H_2O$ when making Fe-solution.

A.2 GENERAL SOLUTIONS

A.2.1 General stock solutions

- **1 N NaOH**

NaOH (Saarchem)	4 g
water to	100 ml

Store in a plastic bottle.

- **1 M HCl**

37% HCl (Saarchem)	8.4 ml
water to	100 ml

Store in a foil covered glass bottle.

- **0.5 M EDTA**

Na_2EDTA (Saarchem)	186.1 g
water to	800 ml

The pH was adjusted to 8.0 with NaOH pellets prior to making the volume to 1 l with water and autoclaving.

- **1 M Tris-HCl**

Tris base	121.5 g
water	800 ml

The pH was adjusted to 8.0 with HCl prior to making the volume to 1 l with water and autoclaving.

- **25% (w/v) Sodium dodecyl sulphate (SDS)**

SDS	50 g
sterile water	200 ml

Stir on warm plate to dissolve. Do not autoclave. The concentration of the SDS solution can be altered by altering the amount of SDS added.

- **70% (v/v) Ethanol (EtOH)**

Absolute ethanol	70 ml
water to	100 ml

Do not autoclave.

- **50% (v/v) Glycerol**

glycerol	25 ml
water to	50 ml

- **2 M Sodium phosphate, mono-sodium (NaH_2PO_4)**

$NaH_2PO_4.H_2O$	55.2 g
water to	200 ml

- **2 M Sodium phosphate, di-sodium (Na_2HPO_4)**

$Na_2HPO_4.2H_2O$	71.2 g
water to	200 ml

- **1 M Phosphate buffer (pH 6.8)**

$NaH_2PO_4.H_2O$ (2 M)	39 ml
$Na_2HPO_4.2H_2O$ (2 M)	61 ml
water to	200 ml

- **0.1 M Phosphate buffer**

Phosphate buffer (1 M, pH 6.8)	10 ml
Water to	100 ml

- **DTT (30%)**

  0.3 g DTT added to 1 ml distilled water
  50 µl aliquots were made and stored at -20°C

- **ASB 14 (10%)**

  0.1 g ASB 14 added to 1 ml distilled water
  50 µl aliquots were made and stored at -20°C

A.2.2 Solutions for protein extractions

- **Protein extraction buffer**

Tris (0.5 M) pH 7.5	5 ml
EDTA (0.5 m)	200 µl
Triton	1%
β-mercaptoethanol	2%
water to	10 ml

- **0.1 M ammonium acetate in methanol (w/v)**

  3.85 g ammonium acetate added to 500 ml of methanol

- **80% acetone (v/v)**

  80ml of acetone added to 20ml of water

- **Urea Lysis Buffer (ULB)**

Urea (8 M)	24.0 g

Dissolve in 25 ml water by stirring and then add:

CHAPS (2 %) (Calbiochem)	0.25 g
Thiourea (Sigma-Aldrich)	7.6 g

Make up to 50 ml with distilled water and filter sterilize.

A.2.3 Solutions for the modified Bradford assay for determining protein concentration

- **Bovine serum albumin (BSA) (1 mg/ml^{-1})**

BSA (Roche)	0.01 g
ULB to	10 ml

- **Bradfords reagent**

Dye Reagent Concentrate (Bio-Rad)	1 ml
sterile water to	5 ml

Make up fresh before every use

- **0.1 M HCl**

HCl	10 ml
Water to	100 ml

A.2.4 Solutions for SDS-PAGE

- **Stacking gel**

4X Tris-Cl / SDS solution (pH 6.8)

Tris base	6.05 g
SDS	0.4 g

Adjust pH to 6.8 with HCl
Add water to100 ml
Filter-sterilize and store at room temperature

Separating gel

4X Tris-Cl / SDS solution (pH 8.8)

Tris base (1.25 M)	91.0 g
SDS	2.0 g

Adjust pH to 8.8 with 1 M HCl
Add water to 500 ml
Filter-sterilize and store at room temperature

- **10 x SDS-PAGE running buffer**

Glycine	150 g
Tris	30 g
SDS	20 g
water to	1000 ml

- **1x SDS PAGE running buffer**

  Dilute 10 x SDS-PAGE running buffer 1:10 with water before use

- **10% (w/v) Ammonium persulfate (AMPS)**

AMPS	1 g
Water to	10 ml

Do not autoclave. Filter sterilize before aliquoting into sterile microfuge tubes and store at -20°C.

- **5x SDS-PAGE Sample Application Buffer (SAB)**

Tris-Cl (pH 6.8)	250 mM
DTT (Promega)	500 mM
SDS (Sigma)	10%
Bromophenol blue (Saarchem)	0.5%
Glycerol	10 ml
water to	5 ml

A.2.5 Solutions for Coomassie staining of polyacrylamide gels

- **Coomassie SDS-PAGE gel stain**

Coomassie brilliant blue R250 (Sigma)	1 g
Methanol (50%)	225 ml
Glacial acetic acid (10%)	50 ml
water to	

Dissolve the Coomassie Brilliant Blue R in methanol before adding acetic acid and water 500 ml

- **Coomassie SDS-PAGE destain**

Methanol	50 ml
Glacial acetic acid	70 ml
water to	1000 ml

- **SDS-PAGE Gel storage solution**

Glacial acetic acid	7 ml
Water to	100 ml

A.2.6 Solutions for 2-DE

- **Rehydration solution**

ULB	X ml
DTT	2%
ASB 14	0.4%
Biolyte 3-10 ampholytes (20%)	1%
Trace amount of bromophenol blue	
Protein sample (250 µg)	Y µl

Made in a total volume of 140 µl.

- **Equilibration buffer I (EQB I)**

Urea (6M)	72.1 g
Tris-HCl (pH 8.8, 0.375 M)	50 ml
Glycerol	20%
SDS	2%
Bromophenol blue	0.0005%

Made up to 200 ml with distilled water, aliquoted into 10 ml volumes and stored at -20°C.

0.1 g DTT was added to 10 ml of EQB I prior to the equilibration step II.

- **Equilibration buffer II (EQB II)**

Made up as for EQB I except that 0.48g of iodoacetamide was added to EQB II prior to equilibration step I.

- **0.5% Agarose**

0.5 g agarose in 1 X SDS running buffer with a trace amount of bromophenol blue.

A.2.7 Western Blot solutions

- **Ponceau S**

Ponceau S powder	0.1%
Ethanol	5%
Made in distilled water	

This stain is re-usuable but due to its sensitivity to light needs to be covered in foil and stored and used in the dark.

- **10X TBS Stock**

50 mM Tris	30.25g
150 Mm NaCl	43.8g

Distilled water to 300 ml
Adjust pH to 7.4 with HCl
Distilled water to 500ml
Autoclave

- **Blocking buffer (5 % skim milk)**

Skim-milk (Elite)	5 g
10 X TBS (1 X)	10 ml
Water to	100 ml

- **TBST (0.1 % Tween in 1X TBS)**

Tween 20 (Saarchem)	0.1 ml
10X TBS (1 X)	100 ml
Water to	1000 ml

Make up fresh
Do not autoclave

- **Towbin Buffer**

Tris base	3.03 g
Glycine (Merck)	14.42 g
Methanol (20%) (Merck)	200 ml
Water to	1000 ml