REFERENCES
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Chapter 8

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APPENDIX A
BUFFERS AND REAGENTS

Milli-Q purified water was used for the preparation of all buffers.

Buffers and reagents for biochemical assay

Phosphate-Buffered Saline (10x, pH 7.0)

80.0 g NaCl, 11.6 g Na₂HPO₄, 2.0 g KH₂PO₄ and 2.0 g KCl was dissolved in 900 ml distilled water, pH was adjusted to 7.0 (Freshly prepare or use within 3 days of preparation, with 2-8°C storage).

Sodium pyrophosphate buffer (0.052 M, pH 8.2)

1.382 g of sodium pyrophosphate was dissolved in 1000 ml of distilled water.

Sodium phosphate buffer (0.10 M, pH 7.6)

0.798 g of Na₂HPO₄ and 0.78 g of NaH₂PO₄ was dissolved in 50 ml of distilled water each. Then, 80.2 ml of Na₂HPO₄ was added to 19.8 ml NaH₂PO₄ and mixed thoroughly.

Phosphate buffer (0.1 M, pH 7)

1.7418 g of K₂HPO₄ and 0.6845 g of KH₂PO₄ was dissolved in 100 ml and 50 ml of distilled water respectively. Then, 61.5 ml of K₂HPO₄ (0.1 M) was added in 38.5 ml of KH₂PO₄ and mixed thoroughly.

Sodium carbonate bicarbonate buffer (pH 10)

0.524 g of Na₂CO₃ and 0.425 g of NaHCO₃ was dissolved in 50 ml of distilled water each. Then, 35 ml of Na₂CO₃ and 15 ml of NaHCO₃ was mixed thoroughly.

TTE buffer (10 mM, pH 7.4)

10 mM of tris was dissolved water and pH was adjusted to 7.4, using HCl. To this 0.05 μl of EDTA (1mM) and 20 μl TritonX100 (0.2 %) was added.
Appendix A

Buffers and Reagents

Tris buffer (0.2 M, pH 8.2)

Tris buffer was prepared by dissolving 24.2 g of tris in distilled water adding 100 ml of EDTA-Na₂ (0.2 M) and volume was made up to 1000 ml with distilled water.

Tripyridyl-s-triazine (TPTZ), (10mM)

7.8 mg of TPTZ was dissolved in 2.5 ml of HCl.

DTNB (10 mM)

99 mg of DTNB was added in 25 ml of absolute methanol.

Phosphate buffer saline (PBS, pH 7.0)

80 g of NaCl, 11.6 g of Na₂HPO₄, 2 g of KH₂PO₄ and 2 g of KCl was added in 10 liter of distilled water. pH was adjusted to 7.0.

TBA (0.67%)

0.67 g of TBA was added in 100 ml of distilled water.

TCA (10%)

10 ml TCA was added in 90 ml of distilled water.

Bradford reagent

10 mg of Coomassie Brilliant Blue G was added in 5 ml of 95 % ethanol. To this 10 ml of orthophosphoric acid was added and volume was made up to 100 ml by adding distilled water. The above solution was filtered and kept in cool dark place.

NBT (300 μM)

2.45 mg NBT was dissolved in 10 ml of distilled water.

NADH (700 μM)

5.78 mg NADH was dissolved in 10 ml of distilled water.

PMS (186 μM)
0.569 mg PMS was dissolved in 10 ml of distilled water.

**Thiourea (0.25%)**

0.25 g thiourea was dissolved in 100 ml of HCl (0.1 M).

**Glycine buffer (0.2 M)**

0.75 g glycine was added in HCl (5 mM).

**Ferrozine**

20 mg of ferrozine was dissolved in 1 ml of distilled water.

**GSH (30 mM)**

53 mg GSH was dissolved in 8.83 ml of distilled water.

**CDNB (30 mM)**

35 mg of CDNB was dissolved in 3.89 ml of distilled water.

**HTAB (0.5%)**

0.03 g of HTAB was dissolved in 5 ml of phosphate buffer (pH 6).

**O-dinasidine (16 mM)**

19.5 mg of o-dinasidine was dissolved in 5 ml of methanol.

**Dipyridyl solution**

0.04 g of Dipyridyl was added in 20 ml of 30% acetic acid.

**GSSG (1 mM)**

0.02 g of GSSG was dissolved in 15 ml of distilled water.

**NADPH (0.1 mM)**

0.09 g of NADPH was dissolved in 20 ml of distilled water.
p-nitro phenyl phosphate (5 mM)

0.02 g of pNPP was dissolved in 30 ml of Sodium carbonate bicarbonate buffer.

Buffers and Reagents for ELISA

Coating buffer (carbonate-bicarbonate buffer)
0.1 M carbonate buffer, pH 9.6
Stock A: 0.2 M: 21.2 g Na₂CO₃ (anhydrous) per liter
Stock B: 0.2 M: 16.8 g NaHCO₃ per liter H₂O
For pH 9.6 add 80 ml A + 170 ml B + 250 ml H₂O

Diluting buffer for ELISA
0.5 g Tween 20 and 2.5 g bovine serum albumin (BSA; 0.25%) was dissolved in 1 liter PBS.

Wash Buffer
In PBS 0.05 % tween-20 was added (Freshly prepare or use within 3 days of preparation, stored at 2-8°C).

Stop Solution - H₃PO₄ (1 M) or H₂SO₄ (2 N)

Buffers for Western Blotting

Tris-buffered saline (TBS)
100 mM TrisCl, pH 7.5
0.9 % (w/v) NaCl
Store up to several months at 4 ºC

Tween 20/TBS (TTBS)
0.1% (v/v) Tween 20 in Tris-buffered saline
Store up to several months at 4°C

Tris/glycerol sample buffer
Appendix A

Buffers and Reagents

25 ml 0.5 M Tri-Cl, pH 6.8
20 ml glycerol
1 mg bromphenol blue
Add H₂O to 100 ml and mix
Store in 1 ml aliquots up to 6 months at -70°C

Tris/glycine electrophoresis transfer buffer

3.02 g Tris base
14.4 g glycine
20% Methanol
H₂O to 1000 ml
Store up to 1 month at -70°C

Comassie blue G-250 staining solution

200 ml acetic acid (20% final)
1800 ml H₂O
0.5 g Coomassie blue G-250 (0.025 % final)
Mix 1 hr and filter (Whatman no. 1 paper)
Store at room temperature indefinitely.

Buffers and media for cell culture

RPMI medium

Powdered RPMI-1640 medium
100 U/ml of penicillin G and
100 µg/ml of streptomycin
2 g of sodium bicarbonate
pH was adjusted to 7.2 and the volume made up to 1 liter with DDW. This was sterile filtered through 0.22 membrane filter using Millipore filtration unit.

Trypan blue, 0.1% (w/v)

Dilute 1 ml commercially available 0.4% (w/v) trypan blue solution (Sigma) with
Appendix A

Buffers and Reagents

3 ml PBS (see recipe) or balanced salt solution (Sigma). Store tightly closed up to 6 months at room temperature.

Giemsa stain

In 100 g Giemsa powder 54 ml glycerol was added and mixture was heated for 2-3 hours at 56 °C. Mixture was cooled down and 840 ml methanol was added. Mixture was allowed to stand at room temperature for 48 h. Stain was filtered and kept at cool and dark place.

Buffers for PFGE

Lysis buffer

1 mg/ml proteinase K,
1 % N-lauroylsarcosine-sodium salt and
EDTA 100 mM (pH 8.0)
Make up volume to 1000 ml

TAE Buffer (50X, pH 8)

24.2 g of tris was dissolved in 50 ml of distilled water. To this, 10 ml of 0.5 M EDTA and 5.71 ml glacial acetic acid were added. Volume was adjusted upto 100 ml by distilled water.
APPENDIX B
PUBLICATIONS
