APPENDIX F

Reagents for proteomics

1. Standard cell lysis buffer

The standard cell lysis buffer consisted of the following ingredients:

Reagent	Quantity	Final concentration
Tris (1M not pH'd)	0.3 ml	30 mM
Thiourea (MW 76.12)	1.522 g	2 M
Urea (MW 60.06)	4.20 g	7 M
CHAPS (MW 614.89)	0.4 g	4% (w/v)
IPG buffer 3–10	0.2 ml	2% (v/v)
HCl (0.1 M)		

All reagents were mixed and dissolved before the pH was adjusted to 8.5 with the 0.1 M HCl. The final volume was made up to 100 ml with distilled water. The buffer was filtered through 0.45 μm membrane filter and kept in small aliquots at –20°C, for up to 3 months.

2. Stock sample/rehydration buffer stock

The stock sample/rehydration buffer (2x) consisted of the following ingredients:

Reagent	Quantity	Final concentration
Urea (MW 60.06)	10.5 g	7 M
Thiourea (MW 76.12)	3.8 g	2 M
CHAPS (MW 614.89)	1 g	4% (w/v)

The reagents were dissolved in 25 ml of DW. It was was filtered through a 0.45 μm sterile membrane filter and kept in small aliquots (2.5 ml) at –20ºC, for up to 6 months.

3. Rehydration buffer

The rehydration buffer consisted of the following ingredients:

Reagent	Quantity	Final concentration
Stock sample/rehydration buffer	2.5 ml	7 M
IPG buffer™	12.5 µl	0.5% (v/v)
DTT (MW 154.2)	7 mg	0.28% (w/v) (0.018 M)

The solution was freshly prepared before use.

4. Stock SDS equilibration buffer solution

The stock SDS equilibration buffer solution consisted of the following ingredients:

Reagent	Quantity	Final concentration
Tris (1.0 M, pH 8.0)	20 ml	100 mM
Urea (MW 60.06)	72.07 g	6 M
Glycerol (87% [v/v], MW 92.09)	69 ml	30% (v/v)
SDS (MW 288.38)	4 g	2% (w/v)

The volume was made up to 200 ml with deionized distilled water. This stock solution could be stored at 25°C for 6 months.

4.1 Equilibration solution 1

The solution was prepared by dissolving 0.5 g of DTT in 100 ml of stock SDS equilibration buffer solution. The solution was freshly prepared just before use.

4.2 Equilibration solution 2

The solution was prepared by dissolving 0.5 g of IAA in stock SDS equilibration buffer solution. It was freshly prepared just before used.

5. Gel preparation and 2–D gels electrophresis

5.1 Tris, pH 8.8 (1.5 M)

Tris base (30.3) g was dissolved in 500 ml of deionized distilled water then the pH was adjusted to 8.8 with 6 N HCl. The final volume was brought up to 1,000 ml with deionized distilled water and the pH was adjusted to pH 8.8. The solution was filtered through a 0.45 μm strerile membrane and stored at 4 ^{o}C for not more than 1 month.

5.2 Sodium dodecyl sulfate (10% SDS; w/v)

This solution was prepared by dissolving 10 g of SDS (Bio-Rad, Hercules, California, USA) in 100 ml of deionized distilled water.

5.3 Ammonium persulfate (10%; w/v)

This solution was prepared just before use by dissolving 1 g of ammonium persulfate (Bio-Rad, Hercules, California, USA) in 10 ml deionized distilled water.

5.4 Water saturated butanol

The solution was prepared by mixing 50 ml of butanol and 50 ml of DW. Once completely separated, the top layer was used to overlay the polyacrylamide gels. The solution was store at 25°C for not longer than 6 months.

5.5 Polyacrylamide gel (12.5%)

The separating polyacrylamide gel (12.5%) was prepared by mixing the following ingredients together:

Reagent	**Quantity for 100 ml of a 12.5% gel**
Acrylamide/Bis 30% (w/v)	41.66 ml
Tris (1.5 M, pH 8.8)	25 ml
ammoniumpersulfade (10% w/v)	500 μl
TEMED (10% v/v)	50 μl

The acrylamide/Bis solution and the Tris buffer were mixed and the final volume was adjusted to 100 ml with deionized distilled water. The preparation was gently mixed and degassed under a vacuum for at least 15 minutes. Prior to addition of ammoniumpersulfade and TEMED, the solution was filtered through a 0.2 μm sterile filter into a clean bottle. The solution was allowed to warm to 25°C prior to addition of ammoniumpersulfade and TEMED and the gel was poured immediately into a casting apparatus.

5.6 Gel storage solution

The gel storage solution consisted of the following ingredients:

Reagent	**Volume**
1.5 M Tris-HCl, pH 8.8	50 ml
10% SDS	2 ml

The final volume was adjusted to 200 ml with deionized distilled water.

5.7 SDS electrophoresis running buffer

The buffer contained the following reagents:

Reagent	**Quantity**	**Final concentration**
Tris (MW 121.14)	30.3 g	25 mM
Glycine (MW 75.07)	144 g	192 mM

SDS (MW 288.38)	10 g	0.1% (w/v)

The final volume was made up to 10 liters with deionized distilled water. The buffer was stored at 25°C for not longer than 3 months.

5.8 Agarose overlay solution (0.5% w/v)

Reagent	**Quantity**	**Final concentration**
SDS electrophoresis running buffer	100 ml	–
Low melting point agarose	0.5 g	0.5% (w/v)
Bromophenol blue	0.2 ml	0.002%

The components were mixed in a 250 ml conical flask and heated on a low setting in the microwave for 1 minute. Ensure all the agarose has melted. The solution was allowed to cool slightly before use. It can be stored at 25°C not for more than 1 month.

5.9 Coomassie Brilliant Blue R-250 stain

5.9.1 Dye solution

Coomasie® Brilliant Blue R-250 dye (Sigma Chemical Co., U.S.A.) (2.5 g) was dissolved in 454 ml of absolute methanol before 92 ml of glacial acetic acid and 454 ml of UDW were added. This dye was filtered through a Whatman No. 1 paper and kept at roo temperature.

5.9.2 Destainin solution

A. High methanol destain

The solution was prepared by mixing 75 ml of gracial acetic acid, 454 ml of methanol and 25 ml of glycerol together. UDW was added to make 1,000 ml. The solution was kept at room temperature.

B. Standard (low-methanol) destain

The solution was prepared by mixing 75 ml of glacial acetic acid, 50 ml of methanol and 25 ml of glycerol together. UDW was added to make 1,000 ml. The solution was kept at room temperature.

5.10 Colloidal Coomassie Brilliant Blue G-250 stain

5.10.1 Fixing solution

The solution was freshly prepared by mixing 10 ml of 85% *o*-phosphoric acid and 20 ml of methanol. The final volume was made to 100 ml with deionized distilled water.

5.10.2 Stock staining solution A

The solution was prepared by dissolving 4 g of ammonium sulfate in 20 ml of deionized distilled water then 0.95 ml of 85% *o*-phosphoric acid was added. The final volume was made to 40 ml with deionized distilled water.

5.10.3 Stock staining solution B

The solution was prepared by dissolving 0.5 g of Coomassie Brilliant Blue G-250 in 1 ml of deionized distilled water.

5.10.4 Staining solution

The solution was freshly prepared by mixing 1 ml of stock staining solution B with 40 ml of stock staining solution A. Then 10 ml of methanol was added and mixed.

5.10.5 Neutralization solution

The solution was prepared by dissolving 6 g of Tris-base in 250 ml of deionized distilled water. The pH was adjusted to 6.5 with *o*-phosphoric acid and the final volume was brought up to 500 ml with deionized distilled water.

5.10.6 Washing solution

The solution was prepared by adding 125 ml of methanol in 375 ml of deionized distilled water.

5.10.7 Stabilizing solution

The solution was prepared by dissolving 100 g of ammonium sulfate in 250 ml of deionized distilled water. The final volume was brought up to 500 ml with deionized distilled water

5.11 Silver stain (Commercial kit: Amersham Biosciences, Sweden)

5.11.1 Fixing Solution

The solution was prepared by adding 100 ml of ethanol and 25 ml of acetic acid in 375 ml of deionized distilled water.

5.11.2 Sensitizing solution

The solution was prepared by dissolving 17 g of sodium acetate in 125 ml of deionized distilled water. Then, 75 ml of ethanol, 1.25 ml of glutaraldehyde (25%

w/v) and 10 ml of sodium thiosulfate (5% w/v) were added. The volume was made up to 250 ml with deionized distilled water.

5.11.3 Silver solution

The solution was prepared by adding 25 ml of silver nitrate solution (2.5% w/v) and 0.1 ml of formaldehyde (37% w/v) in 125 ml deionized distilled water. The volume was made up to 250 ml with deionized distilled water.

5.11.4 Developing solution

The solution was prepared by dissolving 6.25 g of sodium carbonate in 125 ml of deionized distilled water then 0.05 ml of formaldehyde (37% w/v) was added. The volume was made up to 250 ml with deionized distilled water.

5.11.5 Stop solution

The solution was prepared by dissolving 3.65 g of EDTA-Na_2-$2H_2O$ in 125 ml of deionized distilled water then the volume was made up to 250 ml with deionized distilled water.