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APPENDICES

APPENDICES

Reagent for proteomic analysis

1. Reagent for isoelectricfocusing IPGphor

1.1 Sample preparation solution (with urea and thiourea) for 2-D electrophoresis

(7M urea, 2M thiourea, 4% CHAPS, 2% IPG Buffer (carrier ampholytes), 40mM DTT, 25 mL)

Urea		10.5	g
Thiourea		3.8	g
CHAPS		1.0	g
IPG Buffer		500	ml
DTT		154	mg
DI	to	25	ml

Store in 2.5 ml aliqouts at -20 °C.

1.2 1M DTT

DTT		1.5425	g
DI	to	10	ml

Store in 2.5 ml aliqouts at -20 °C.

1.3 Thiourea rehydration stock solution

(7M urea, 2M thiourea, 2% CHAPS, 0.5% Pharmalyte or IPG Buffer, 0.002% bromophenol blue, 25 mL)

Urea		0.5	g
Thiourea		3.8	g
CHAPS 2%		0.5	g
IPGphor 0.5%		125	µL
1% Bromophenol blue stock solution 0.002%		50	µL
DI	to	25	µL

Store in 1.0 mL aliquots at -20 °C.

1M DTT is added just prior to use. Add 1M DTT 18.6 µL per 1.0 mL aliquot of rehydration stock solution.

2. Reagents for equilibration buffer of IPG strip for SDS-PAGE

2.1 SDS equilibration buffer solution

(6 M urea, 75 mmM Tris-HCL PpH 8.8, 29.3% GLYCEROL, 2 % SDS, 0.002% bromophenol blue, 200 mL)*

Urea		72.1	g
Tris-HCL, pH8.8		10.0	µL
Glycerol		69	ml
SDS		4.0	g
1% Bromophenol blue stock solution		400	µL
DI	to	200	ml

Store in 20 mLaliquots at -20 °C.

*This in a stock solution. Just prior to use, add DTT to one portion (100 mg per 10 mL) and iodoacetamide to the other (250 mg per 10 ml).

3. Reagent for SDS polyacrylamide gel electrophoresis

3.1 10X Laemmli SDS electrophoresis buffer

(250 Mm Tris base, 1.92 M glycine, 1% SDS, 10 L)*

Tris base		303	g
Glycine		1441	g
SDS		100	g
Double-distilled water (DDW)	to	10	µL

The pH of this solution should not be adjusted.

Store at room temperature.

3.2 30%T, 2.6 C monomer stock solution

(30% acrylamide, 0.8% N,N'-methylenebisacrylamide, 1L)

Acrylamide		300	g
N,N'-methylenebisacrylamide		8	g
DI	to	1	L

Filter solution through a 0.45 μm filter. Store at 4 °C in the dark.

3.3 4X resolving gel buffer solution

(1.5 M Tris base, pH 8.8, 1L)

Tris base		181.7	g
DDW		750	mL
HCl		adjust to pH 8.8	
DI	to	1	L

Filter solution through a 0.45 μm filter. Store at 4 °C.

3.4 Bromophenol blue stock solution

Bromophenol blue		100	mg
Tris-base		60	mg
DI	to	10	mL

3.5 10% SDS solution

(10% SDS, 50 mL)

SDS		5.0	g
DI	to	50	μL

Filter solution through a 0.45 μm filter. Store at room temperature.

3.6 10% ammonium persulfate

Ammonium persulfate	0.05	g
DI	0.5	mL

Prepared just prior to use.

3.7 Gel storage solution

(375 mM Tris-HCL, 0.1% SDS, 1L)

4X resolving gel buffer		250	mL
10% SDS		10	mL
DDW	to	1	L

Store at 4 °C.

3.8 12% gel recipes for miniVE sysems

Final gel concentration	12%
Mono solution (mL)	4.0
4X resolving gel buffer (mL)	2.5
10% SDS (mL)	0.1
DI (mL)	3.35
10% ammonium persulfate (µL)	50
TEMED (µL)	3.3
Total volume (mL)	10

A de-aeration step may be performed about 10 min. Ammonium persulfate and TEMED are added immediately prior to casting the gel. Gently swirl the flask to mix, being careful not to generate bubbles. Immediately pour the gel.

3.9 1X Laemmli SDS electrophoresis buffer

(25mM Tris base, 192 Mm glycine, 0.1% SDS, 10 L)*

Tris base		30.3	g
Glycine		144.0	g
SDS		10.0	g
DDW	to	10	L

This solution can be prepared by diluting one volume of 10 x Laemmli SDS buffer with nine volumes of double-distilled water. Store at room emperature.

3.10 Agarose sealing solution

(25mM Tris base , 192mM glycine, 0.1% SDS, 0.5% agaroe, 0.002% bromophenaol blue, 100 mL)

Laemmli SDS electrophoresis buffer	100	mL
Agarose	0.5	g
1% Bromophenol blue stock solution	200	µL

4. Reagent for Coomassie staining

4.1 Trichloroacetic acid fixing solution (for IEF gels)

(20% (w/v) trichloroacetic acid, 500 mL)

Trichloroacetic acid		100	g
DI	to	500	mL

Store up to 1 month at room temperature.

4.2 Coomassie Blue staining solution

(0.025% Coomassie Brilliant Blue R-250, 40% (v/v) methanol, 7% (v/v), acetic acid, 2L)

Coomassie Brilliant Blue R-250		0.5	g
Methanol		800	mL

Stir until dissolved. Filter. Then add:

Acetic acid		140	mL
DI	to	2	L

Store at room temperature for up to 6 months.

4.3 Destain solution I (40% (v/v) methanol, 7%(v/v) acetic acid, 1L)

Methanol		400	mL
Acetic acid		70	mL
DI	to	1	L

Store at room temperature.

4.4 **Destain solution II** (7% (v/v), acetic acid, 5% (v/v) methanol, 10 L)

Acetic acid		700	mL
Methanol		500	mL
DI	to	10	L

Store at room temperature.

RESEARCH PUBLICATION

1. PUBLICATION

Jumpajan J, Prasongwattana V, Sriboolue P, Wongkham C , Thongboonkerd V. **Proteomic Analysis of Renal Cortex, A Comparative Study Between Non-Potassium-depleted and Potassium-depleted Northeastern Thais**. Srinagarind Med J. 2009; 24 (suppl): 134-137 (proceeding).

2. PRESENTATION

Jumpajan J, Prasongwattana V, Sriboolue P P, Wongkham C , Thongboonkerd V. **Proteomic Analysis of Renal Cortex, A Comparative Study Between Non-Potassium-depleted and Potassium-depleted Northeastern Thais**. People-Centered Health Care in Economic Crisis Era, Faculty of Medicine, Khon Kaen University. October 13-16, 2009 (poster presentation).

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