



APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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APPENDIX A

Reagents and buffer preparation

Reagents for density gradient ultracentrifugation

1. Density solution 1.006 g/mL (solution A)

- NaCl 22.8 g
- Na₂EDTA 0.2 g
- NaOH (1mol/L) 2 ml
- Double distilled H₂O to 2 L

2. Density solution 1.019 g/mL

- KBr 0.376 g
- solution A to 20 ml

3. Density solution 1.063 g/mL

- KBr 1.682 g
- solution A to 20 ml

4. Density solution 1.24 g/mL

- KBr 7.497 g
- solution A to 20 ml

Reagents for dialysis**1. 10X PBS buffer, pH 7.2**

- NaCl	80	g
- KCl	2	g
- Na ₂ HPO ₄ (MW 141.96)	11.5	g
- KH ₂ PO ₄ (MW 174.18)	2	g
- Double distilled H ₂ O to	1,000	ml

Reagents for Lowry protein assay**1. Reagent A**

- CTC	5	ml
- 20% Na ₂ CO ₃	5	ml
- 0.8 N NaOH	10	ml
- 5% SDS	20	ml

2. Reagent B

- Folin-Ciocalteu phenol reagent	1	ml
- Sterile water	5	ml

3. Stock solution CTC

- CuSO ₄ ·7H ₂ O	0.2	g
- Tatalic acid	0.4	g
- Sterile water to	100	ml

4. 0.8 N NaOH

- NaOH	1.6	g
- Sterile water to	50	ml

5. 5% SDS

- SDS 2.5 g
- Sterile water to 50 ml

6. 20% Na₂CO₃

- Na₂CO₃ 10 g
- Sterile water to 50 ml

Reagents for one-dimensional electrophoresis**1. 12.5% acrylamide solution**

- 40% acrylamide solution 3.125 ml
- 1.5 M Tris-HCl pH 8.8 2.5 ml
- 10% SDS 125 µl
- Double distilled water 4.225 ml
- 10% APS 50 µl
- TEMED 3.3 µl

2. stacking solution

- 40% acrylamide solution 300 µl
- 0.5 M Tris-HCl pH 6.8 742 µl
- 10% SDS 30 µl
- Double distilled water 1.916 ml
- 10% APS 23 µl
- TEMED 1.7 µl

3. 1.5 M Tris-HCl pH 8.8

- Tris 36.3 g
- HCl to pH 8.8
- Double distilled water to 200 ml

4. 0.5 M Tris-HCl pH 6.8

- Tris 3 g
- HCl to pH 6.8
- Double distilled water to 50 ml

5. 10% SDS solution

- SDS 10 g
- Double distilled water to 100 ml

6. SDS electrophoresis buffer

- Tris 3.02 g
- Glycine 14.4 g
- SDS 1 g
- Double distilled water to 1000 ml

7. 10% ammonium persulfate solution (10% APS)

- Ammonium persulfate 0.1 g
- Double distilled water to 1 ml

8. Silver staining reagents

8.1 Fixing solution

- 50% Methanol 500 ml
- 12% Acetic acid 120 ml
- 37% Formaldehyde 500 μ l
- Double distilled water to 1000 ml

8.2 Washing solution

- Ethanol 350 ml
- Double distilled water to 1000 ml

8.3 Sensitizing solution

- Sodium thiosulfate 0.2 g
- Double distilled water to 1000 ml

8.4 Staining solution

- Silver nitrate 2 g
- Double distilled water to 1000 ml

8.5 Developing solution

- Sodium carbonate 60 g
- 0.02% Sodium thiosulfate 20 ml
- 37% Formaldehyde 500 μ l

- Double distilled water to 1000 ml

8.6 Stopping solution

- Sodium-EDTA 14.6 g
- Double distilled water to 1000 ml

8.7 Storing solution

- Acetic acid 1 ml
- Double distilled to 1000 ml

Reagent for two-dimension electrophoresids**1. Lysis solution (7M urea, 2M thiourea, 4% CHAPS)**

- Urea 4.2 g
- Thiourea 1.5 g
- CHAPS 0.4 g
- Double distilled water to 10 ml

2. Rehydration stock solution without IPG buffer

- Urea 4.2 g
- Thiourea 1.5 g
- CHAPS 0.4 g
- Bromphenol blue 20 μ l of 1% solution
- Double distilled water to 10 ml

3. SDS equilibration buffer

- Tris-HCl pH 8.8 10 ml
- Urea 72.07 g
- Glycerol 69 ml
- SDS 4 g
- Bromphenol blue 400 μ l of 1% solution
- Double distilled water to 200 ml

4. Agarose sealing solution

- SDS electrophoresis buffer 10 ml
- Agarose 0.05 g
- Bromphenol blue 20 μ l of 1% solution

5. Bromphenol blue stock solution

- Bromphenol blue 10 mg
- Tris-base 6 mg
- Double distilled water to 1 ml

Reagents for In-gel trypsin digestion**1. 20 mM Ammonium bicarbonate**

- Ammonium bicarbonate 79.6 mg
- Sterile water to 50 ml

2. 10 mM DTT/ 10 mM ammonium bicarbonate

- DTT 7.7125 mg
- 10 mM ammonium bicarbonate 5 ml

3. 100 mM Iodoacetamide/ 10 mM ammonium bicarbonate

- Iodoacetamide 92 mg
- 10 mM ammonium bicarbonate 5 ml

4. 10 ng Trypsin in 50% acetonitrile/ 10 mM ammonium bicarbonate

- Trypsin 20 μ g
- 50% acetonitrile/ 10 mM ammonium bicarbonate 2 ml

5. 30% acetonitrile

- 100% acetonitrile 1.5 ml
- Sterile water to 5 ml

6. 0.1% FA

- Formic acid 50 μ l
- Sterile water to 50 ml

7. 50% acetonitrile/ 0.1% FA

- 100% acetonitrile 5 ml
- 0.1% FA 5 ml

APPENDIX B

Condition for MS/MS ions search

MASCOT MS/MS Ions Search

Your name	keeratiporn	Email	sessionalpaper@hotmail.com
Search title	1 b		
Database(s)	Environmental_EST SwissProt NCBItr contaminants cRAP	Enzyme	Trypsin
		Allow up to	1 missed cleavages
		Quantitation	None
Taxonomy Homo sapiens (human)		
Fixed modifications	Carbamidomethyl (C)		Acetyl (K) Acetyl (N-term) Acetyl (Protein N-term) Amidated (C-term) Amidated (Protein C-term) Ammonia-loss (N-term C) Biotin (K) Biotin (N-term) Carbamyl (K) Carbamyl (N-term) Carboxymethyl (C)
	Display all modifications <input type="checkbox"/>		
Variable modifications	Dioxidation (M)		
Peptide tol. ±	1.2 Da	# ¹³ C	0 MS/MS tol. ± 0.6 Da
Peptide charge	1+, 2+ and 3+	Monoisotopic	<input checked="" type="radio"/> Average <input type="radio"/>
Data file	D:\วิทย์พนธ์\biotec\2D 5 march 20... <input type="button" value="เลือก..."/>		
Data format	Mascot generic	Precursor	m/z
Instrument	ESI-TRAP	Error tolerant	<input type="checkbox"/>
Decoy	<input type="checkbox"/>	Report top	50 hits
<input type="button" value="Start Search ..."/>		<input type="button" value="Reset Form"/>	

Figure 28 MASCOT MS/MS ions search

CURRICULUM VITAE

Name Miss Keeratiporn Worrasettasing

Date of Birth February 21, 1987

Education background

Year	Degree	Institution
2005	Certificated of high school	Yothinburana
2009	Bachelor of Science (Medical Technology)	Chiang Mai University

Research support

2011	The NSTDA Research Chair Grant
	The National Research University Project under Thailand's Office of the Higher Education Commission
	PHC Franco-Thai cooperation program in higher education and research
	Graduate school, Chaing Mai University

Honors and Awards

2012	Excellent Oral Presentation at The 1st ASEAN Plus Three Graduate Research Congress (AGRC 2012) at The Empress Hotel, Chiang Mai, Thailand
2009	Excellent Poster Presentation at Faculty of Associated Medical Sciences, Chiang Mai University

Presentations and publications

1. Worrasettasing K, Taneyhill K. Anti-tumor activity of pre-germinated brown rice. 2009. (Poster presentation)
2. Worrasettasing K, Roytrakul S, Kasinrerak W, Taneyhill K. Lipoproteomics of Low Density Lipoprotein Subfractions. Proceedings: 1st ASEAN Plus Three Graduate Research Congress. 2012. (Oral presentation and proceeding)
3. Worrasettasing K, Roytrakul S, Kasinrerak W, Taneyhill K. Lipoproteomics of Low Density Lipoprotein Subfractions. The conference on Recent Advances in Diagnosis and Monitoring of Liver Diseases at the Annual Meeting of the Faculty of Associated Medical Sciences, Chiang Mai University. 2012. (Poster presentation)