

## APPENDICES

### Reagent preparations

#### I. Reagent for electrophoresis of DNA on agarose gel

##### 1. 0.5 M EDTA pH 8.0

EDTA	18.61	g
Distilled water	90	ml

Mixed well, adjusted pH to 8.0 with 5 N KOH and then adjusted volume to 100 ml. Sterilized by autoclave and stored at room temperature.

##### 2. 10xTris-Borate-EDTA (TBE) buffer

Tris (hydroxymethyl) aminomethane	108	g
Boric acid	55	g
0.5 mM EDTA	40	ml

Dissolved and adjusted volume to 1,000 ml with distilled water.

##### 3. 6xLoading dye for agarose gel

Glycerol	800	$\mu$ l
0.5 M EDTA	40	$\mu$ l
Bromophenol blue	50	mg

Dissolved and adjusted to 2 ml with sterile distilled water.

##### 4. Ethidium bromide working solution

Ethidium bromide	1.0	g
Distilled water	100	ml

Kept in the dark bottle and stored at room temperature.

## II. Reagent for culture of bacteria

### 1. LB broth

Tryptone	7.5	g
Yeast extracts	3.75	g
NaCl	7.5	g

Dissolved and adjusted volume to 500 ml with distilled water and autoclaved.

### 2. LB agar

7.0 g of LB agar were dissolved in 200 ml distilled water and autoclaved.

## III. Reagent for electrophoresis on SDS-PAGE and Western blotting.

### 1. Separating gel buffer stock (1.5 M Tris-HCl pH 8.8)

36.6 g of Tris base were dissolved in approximately 150 ml deionized water and then adjusted to pH 8.8 with HCl. Made volume to 200 ml with deionized water and stored at 4 °C.

### 2. Stacking gel buffer stock (0.5 M Tris-HCl pH 6.8)

12.0 g of Tris base were dissolved in approximately 60 ml deionized water and then adjusted to pH 6.8 with HCl. Made volume to 100 ml with deionized water and stored at 4 °C.

### 3. 2xSDS-PAGE loading dye

Stacking gel buffer stock pH 6.8	12.5	g
SDS	2	g
Glycerol	0.005	g
Bromphenol blue	10	ml

Dissolved and adjusted volume to 50 ml with deionized water.

Aliquoted and stored at -20 °C.

**4. 30% Acrylamide stock solution**

Acrylamide	60.0 g
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N’N’-bis-methylene-acrylamide (Bis)	1.6 g
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Dissolved in about 150 ml deionized water then adjusts to 200 ml with deionized water. Stored at 4 °C.

**5. 10% Ammonium persulfate**

Dissolved ammonium persulfate 0.1 g and made volume to 1 ml with deionized water.

**6. 10% SDS**

Dissolved SDS (Sodium dodesyl sulfate) 10 g and made volume to 100 ml with deionized water

**7. 10xTank buffer (stock)**

Glycine	144.0 g
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SDS	10.0 g
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Dissolved and adjusted volume to 1,000 ml with deionized water. No need to adjust pH with acid or base.

**8. Working Tank buffer**

To make 1 liter of 1x electrophoresis buffer (0.025 M Tris, 0.192 M Glycine, 0.1%SDS, pH 8.3) diluted 100 ml of 10xTank buffer with 900 ml deionized water.

**9. 10x Transferring buffer stock**

Tris base	30.3 g
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Glycine	141.4 g
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Dissolved and adjust to 1,000 ml with deionized water.

## 10. Working Transferring buffer

To make 1 liter of working transferring buffer diluted 100 ml of 10xTransferring buffer with added 200 ml of methanol. Bring to 1 liter with deionized water. Do not adjust the pH, which should be between 8.2 and 8.4.

## 11. 10xTBS-Tween buffer pH 7.5

Tris base	60	g
NaCl	90	g

Dissolved with deionized water approximately 600 ml, adjusted pH to 7.5 and filled deionized water to volume 1,000 ml. After that added 5 ml of Tween- 20, mixed and stored at room temperature.

## 12. Working TBS-Tween buffer pH 7.5

To make 1 liter of 1x TBS-Tween buffer pH 7.5 diluted 100 ml of 10xTransferring buffer with 900 ml deionized water.

## 13. Coomassie blue staining

### 13.1 Coomassie Blue staining solution

Coomassie Brilliant Blue R250	0.125	g
Methanol	200	ml
Acetic acid	35	ml

Mixed and adjusted volume to 500 ml with deionized water. Stored at room temperature.

### 13.2 Destain I

Methanol	200	ml
Acetic acid	70	ml

Mixed and adjusted volume to 500 ml with deionized water. Stored at

room temperature.

### 13.3 Destain II

Methanol	50	ml
Acetic acid	70	ml

Mixed and adjusted volume to 500 ml with deionized water. Stored at room temperature.

### 14. Ponceau S staining

#### 14.1 Ponceau S 0.5% (w/v) solution

Ponceau S	1.5	g
Trichloroacetic acid (TCA)	10.5	g
Sulfosalicylic acid	10.5	g

Dissolved and adjust to 300 ml with distilled water.

#### 14.2 Destain solution

Distilled water	285	ml
Acetic acid	15	ml

Mixed and stored at room temperature.

### 15. 5% skimmed milk blocking buffer for Western blotting

Dissolved skimmed milk 5 g made to 100 ml with TBS-Tween pH 7.5

## IV. Reagent for affinity chromatography to purified (His)<sub>6</sub>-survivin fusion protein

### 1. 4xBinding buffer

NaCl	116	g
Tris-HCl	9.6	g
Imidazole	1.4	g

Dissolved with deionized water approximately 800 ml, adjusted pH to 7.9 with HCl and filled deionized water to volume of 1,000 ml.

## 2. 1xBinding buffer for denaturing condition (5 mM Imidazole)

4xbinding buffer	25	ml
Urea	36	g

Dissolved with deionized water to approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

## 3. Washing buffer without Imidazole

NaCl	2.9	g
Tris-HCl	0.24	g
Urea	36	g

Dissolved with deionized water to volume approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

## 4. Washing buffer (20 mM Imidazole)

NaCl	2.9	g
Tris-HCl	0.24	g
Imidazole	0.14	g
Urea	36	g

Dissolved with deionized water to volume approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

## 5. Washing buffer (40 mM Imidazole)

NaCl	2.9	g
Tris-HCl	0.24	g
Imidazole	0.27	g

Urea	36	g
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Dissolved with deionized water to volume approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

#### 6. Washing buffer (60 mM Imidazole)

NaCl	2.9	g
Tris-HCl	0.24	g
Imidazole	0.41	g
Urea	36	g

Dissolved with deionized water to volume approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

#### 7. Washing buffer (80 mM Imidazole)

NaCl	2.9	g
Tris-HCl	0.24	g
Imidazole	0.55	g
Urea	36	g

Dissolved with deionized water to volume approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

#### 8. 1x Elution buffer (500 mM Imidazole)

NaCl	2.9	g
Tris-HCl	0.24	g
Imidazole	3.4	g

Dissolved with deionized water to volume approximately 90 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

**9. 4x Striping buffer**

NaCl	11.6 g
Tris-HCl	0.96 g
ETDA	14.88 g

Dissolved with deionized water to volume approximately 80 ml, adjusted pH to 7.9 with HCl and filled with deionized water to volume 100 ml.

**10. 8x Charge buffer**

Dissolved  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$  10.5 g made to 100 ml with deionized water.

**V. Reagent for ELISA****1. 10x PBS pH 7.4**

NaCl	80 g
KCl	2 g
$\text{Na}_2\text{HPO}_4$	11.5 g
$\text{KH}_2\text{PO}_4$	2 g

Dissolved with deionized water approximately 80 ml, may need to adjusted pH to 7.4 with NaOH and filled with deionized water to volume 1,000 ml.

**2. Working PBS-Tween pH 7.4**

To make 1 liter of 1x PBS-Tween buffer pH 7.4 diluted 100 ml of 10xPBS pH 7.4 with 900 ml deionized water and added 500  $\mu\text{l}$  of Tween-20

**3. Carbonate-bicarbonate buffer pH 9.6 (Coating buffer)**

$\text{Na}_2\text{CO}_3$	1.59 g
$\text{NaHCO}_3$	2.93 g



Dissolved in ~ 800 ml deionized water and then adjusted pH to 9.6 with HCl. Added deionized water to 1,000 ml. Stored at 4 °C.

#### 4. 50 mM Tris pH 8.5

Tris 1.211 g

Dissolved in ~ 800 ml deionized water and then adjusted pH to 8.5 with HCl. Added deionized water to 1,000 ml. Stored at 4 °C.

#### 5. 50 mM Succinate buffer pH 5.2

Succinic acid 1.18 g

Dissolved in ~ 800 ml deionized water and then adjusted pH to 5.2 with NaOH. Added deionized water to 1,000 ml. Stored at 4 °C.

#### 6. 5% skimmed milk blocking and antibody dilution buffer

Dissolved skimmed milk 5 g made to 100 ml with PBS-Tween pH 7.4

#### 7. 1% BSA (Bovine Serum Albumin) blocking and antibody dilution buffer

Dissolved BSA 1 g made to 100 ml with PBS-Tween pH 7.4

#### 8. 1 N HCl

37% HCl solution 8.3 ml

Deionized water 91.3 ml

Prepared in fume hood by gradually adding HCl solution into distilled water with gentle stirring and stored at room temperature.

## CURRICULUM VITAE

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1997-2000 Bachelor of Science, B.Sc. (Medical Technology), Chiang Mai University, Chiang Mai, Thailand.

### PUBLICATIONS

1. Saosathan S. Detection of *Helicobacter pylori* from gastric biopsy by Polymerase Chain Reaction. 1999. Research project as part of the B.Sc. course completion.
2. Saosathan S., Chewaskulyoung B., Saeteng S., Lertprasertsuke N., Kasinrerak W. and Cressey R., Expression and Production of Auto-antibodies to Livin and Survivin Anti-Apoptotic Proteins in Lung Cancer. Journal of Medical Technology. 2010 (In press).

**POSTER PRESENTATION**

สวลี เส้าสะท้าน, บุษยามาส ชิวสกุลยง, สมเจริญ แซ่เต็ง, นิรัชร์ เลิศประเสริฐสุข, วัชระ กสิณ  
ฤกษ์ และ รัชดา เกรสซี่ “การแสดงออกและการสร้างอโต้แอนติบอดีต่อโปรตีนด้านอะพอโพโท  
ซิส ไลวินและเซอร์ไววิน ในมะเร็งปอด”, งานวันวิชาการมหาวิทยาลัยเชียงใหม่ วิถีวิจัย: ทศวรรษที่  
5 สู่ความเป็นเลิศ, ณ หอประชุมมหาวิทยาลัยเชียงใหม่, 26-27 พฤศจิกายน 2552.



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