# APPENDIX

## 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. Steriled 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 | µl |
| 0.5 M EDTA | 40 | µl |
| Bromophenol blue | 50 | mg |

Dissolved and adjusted to 2 ml with steriled 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.

### 3. Super broth

| | | |
|---|---|---|
| Tryptone | 15 | g |
| Yeast extract | 7.5 | g |
| MOPS | 5 | g |

Dissolved and adjusted volume to 500 ml with 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)

36.3 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 |
| N'N'-bis-methylene-acrylamide (Bis) | 1.6 | g |

Dissolved in about 150 ml deionized distilled water then adjusts to 200 ml with deionized water. Stored at 4°C in dark.

**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)**

| | | |
|---|---|---|
| Tris base | 30.3 | g |
| Glycine | 144.0 | g |
| SDS | 10.0 | g |

Dissolved and adjusted volume to 1,000 ml with deionized distilled 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 |
| Glycine | 141.4 | g |

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 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 1000 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 Billiant 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. 5% skimmed milk blocking buffer for Western blotting**

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

## IV. Reagent for affinity chromatography to purified $(His)_6$-p53 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 and filled deionized water to volume of 1000 ml.

### 2. Working binding buffer for denaturing condition (20 mM Imidazole)

| | | |
|---|---|---|
| 4xbinding buffer | 25 | g |
| Urea | 36 | g |

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

### 3. 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 and filled with deionized water to volume 100 ml.

### 4. Eluting buffer (1 M Imidazole)

| | | |
|---|---|---|
| NaCl | 2.9 | g |
| Tris-HCl | 0.24 | g |
| Imidazole | 6.8 | g |

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

**5. 4x Striping buffer**

| | | |
|---|---|---|
| NaCl | 11.6 | g |
| Tris-HCl | 0.96 | g |
| EDTA | 14.88 | g |

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

**6. 8x Charge buffer**

Dissolved $NiSO_4.6H_2O$ 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 |
| $Na_2HPO_4$ | 11.5 | g |
| $KH_2PO_4$ | 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 µl of Tween-20

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

| | | |
|---|---|---|
| $Na_2CO_3$ | 1.59 | g |
| $NaHCO_3$ | 2.93 | g |

Dissolve in ~ 800 ml distilled water and then adjusted pH to 9.6 with HCl. Added distilled water to 1000 ml. Stored at 4°C.

**4. 5% skimmed milk blocking and antibody dilution buffer**

Disolved skimmed milk (Mission) 5 g made to 100 ml with PBS-Tween pH 7.4

**5. 3% BSA (Bovine Serum Albumin) blocking and antibody dilution buffer**

Disolved BSA 3 g made to 100 ml with PBS-Tween pH 7.4

**6. 1 N HCl**

| | | |
|---|---|---|
| 37% HCl solution | 8.3 | ml |
| Distilled 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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**PUBLICATIONS**

1. Pimpa S. p53 accumulation and pathological features of colorectal adenocarcinoma. 2003. Research project as part of the B.Sc course completion
2. Cressey R., Pimpa, S. Tontrong W., Watananupong O., Lertprasertsuke N, Expression of cyclooxygenase-2 in colorectal adenocarcinoma is associated with p53 accumulation and hdm2 overexpression. Cancer Lett. 20069; 233(2): 232-9
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