APPENDIX

1. Chemical Preparation

1.1 GSTs assay: 0.1 M potassium phosphate buffer pH 6.5 (homogenization and substrate buffer)

Prepared stock of; 0.5 M potassium dihydrogen phosphate (KH_2PO_4) 500 ml

0.5 M di-potassium hydrogen phosphate (K_2HPO_4) 500 ml

KH_2PO_4 MW = 136.09 M (MERCK)

Thus; 1 M = 136.09 g/l

= 0.1361 g/ml

= 68.05 g/500 ml

0.5 M = 34.025 g/ distilled water 500 ml

K_2HPO_4 MW = 174.18 M (MERCK)

Thus; 1 M = 174.18 g/l

= 0.1742 g/ml

= 87.1 g/500 ml

0.5 M = 43.55 g/ distilled water 500 ml

The 0.5 M KH_2PO_4 was adjusted with 0.5 M K_2HPO_4 into pH 6.5 and kept as stock (0.5 M potassium phosphate buffer pH 6.5). The stock was diluted to 0.1 M potassium phosphate buffer pH 6.5

1.2 GSTs assay: 1 µM DL-dithiothreitol (DTT) (antioxidant in homogenization buffer)

Prepared; 1 mM DTT 1 ml

DTT MW = 154.25 M (Bio Basic Inc.)

Thus; 1 M = 154.25 g/l

= 0.154 g/ml

= 154 mg/ml

10 mM = 1.54 mg/ distilled water 1 ml

Then the 10 mM DTT was diluted to 1 µM DTT in 0.1 M potassium phosphate buffer pH 6.5.

1.3 α- and β-carboxylesterase assays: 20 mM potassium phosphate buffer pH 7.2 (homogenization and substrate buffer)

Prepared stock of; 0.1 M potassium dihydrogen phosphate (KH_2PO_4) 500 ml

0.1 M di-potassium hydrogen phosphate (K_2HPO_4) 500 ml

KH_2PO_4 MW = 136.09 M (MERCK)

Thus; 1 M = 136.09 g/l

= 0.1361 g/ml

= 68.05 g/500 ml

0.1 M = 6.805 g/ distilled water 500 ml

K_2HPO_4 MW = 174.18 M (MERCK)

Thus; 1 M = 174.18 g/l

= 0.1742 g/ml

= 87.1 g/500 ml

0.1 M = 8.71 g/ distilled water 500 ml

The 0.1 M K_2HPO_4 was adjusted with 0.1 M KH_2PO_4 into pH 7.2 and kept as stock (0.1 M potassium phosphate buffer pH 7.2). The stock was diluted to 20 mM potassium phosphate buffer pH 7.2.

1.4 Mixed-function oxidase assay: 0.25 M sodium acetate buffer pH 5.0 (homogenization and substrate buffer)

Prepared; 0.25 M sodium acetate ($C_2H_3NaO_2$) 500 ml

$C_2H_3NaO_2$ MW = 82.03 M (BDH Chemicals Ltd.)

Thus; 1 M = 82.03 g/l

= 0.082 g/ml

= 41 g/500 ml

0.25 M = 10.25 g/ distilled water 500 ml

Dissolved 10.25 g $C_2H_3NaO_2$ in 300 ml distilled water. Adjusted the pH to 5.0 with acetic acid and made up the volumn to 500 ml.

1.5 Mixed-function oxidase assay: 3% hydrogen peroxide

The 10 ml of 3% hydrogen peroxide (H_2O_2) (MERCK) was prepared from 30% H_2O_2 dilution with distilled water.

1.6 Acid phosphatase assay: 50 mM sodium acetate buffer pH 4.0 (homogenization and substrate buffer)

Prepared; 50 mM sodium acetate ($C_2H_3NaO_2$) 500 ml

$C_2H_3NaO_2$ MW = 82.03 M (BDH Chemicals Ltd.)

Thus; 1 M = 82.03 g/l

= 0.082 g/ml

= 41 g/500 ml

50 mM = 2.05 g/ distilled water 500 ml

Dissolved 2.05 g $C_2H_3NaO_2$ in 300 ml distilled water. Adjusted the pH to 4.0 with acetic acid and made up the volume to 500 ml.

1.7 Alkaline phosphatase assay: 50 mM Tris-HCl buffer pH 8.0 (homogenization and substrate buffer)

Prepared; 50 mM Tris-HCl 500 ml

Trizma® base MW = 121.14 M (Sigma)

Thus; 1 M = 121.14 g/l

= 0.121 g/ml

= 60.5 g/500 ml

50 mM = 3.02 g/ distilled water 500 ml

Dissolved 3.02 g Trizma® base in 300 ml distilled water. Adjusted the pH to 8.0 with HCl and made up the volume to 500 ml.

1.8 Alkaline phosphatase assay: 1 mM DL-dithiothreitol (DTT) (antioxidant in homogenization buffer)

Prepared; 1 mM DTT 1 ml

DTT MW = 154.25 M (Bio Basic Inc.)

Thus; 1 M = 154.25 g/l

= 0.154 g/ml

= 154 mg/ml

20 mM = 3.08 mg/ distilled water 1 ml

Then the 20 mM DTT was diluted to 1 mM DTT in 50 mM Tris-HCl buffer pH 8.0.

1.9 Acetylcholinesterase assay: 0.1 M potassium phosphate buffer pH 7.0 (homogenization and substrate buffer)

Prepared stock of; 0.5 M potassium dihydrogen phosphate (KH_2PO_4) 500 ml

0.5 M di-potassium hydrogen phosphate (K_2HPO_4) 500 ml

KH_2PO_4 MW = 136.09 M (MERCK)

Thus; 1 M = 136.09 g/l

= 0.1361 g/ml

= 68.05 g/500 ml

0.5 M = 34.025 g/ distilled water 500 ml

K_2HPO_4 MW = 174.18 M (MERCK)

Thus; 1 M = 174.18 g/l

= 0.1742 g/ml

= 87.1 g/500 ml

0.5 M = 43.55 g/ distilled water 500 ml

The 0.5 M K_2HPO_4 was adjusted with 0.5 M KH_2PO_4 into pH 7.0 and kept as stock (0.5 M potassium phosphate buffer pH 7.0). The stock was diluted to 0.1 M potassium phosphate buffer pH 7.0.

1.10 Acetylcholinesterase assay: 1 µM DL-dithiothreitol (DTT) (antioxidant in homogenization buffer)

Prepared; 1 mM DTT 1 ml

DTT MW = 154.25 M (Bio Basic Inc.)

Thus; 1 M = 154.25 g/l

= 0.154 g/ml

= 154 mg/ml

10 mM = 1.54 mg/ distilled water 1 ml

Then the 10 mM DTT was diluted to 1 µM DTT in 0.1 M potassium phosphate buffer pH 7.0.

1.11 Protein determination assay: Bio Rad (1:4 Dye)

The Bio Rad protein assay reagent (Bio-Rad Laboratories Inc.) was diluted to 1:4 dilution with distilled water.

2. Standard Preparation

2.1 Protein standard (albumin standard stock concentration = 2 mg/ml)

The albumin standard stock (Thermo) was diluted to 0.1-0.5 mg/ml concentration with homogenization buffer of each assay, as follows;

Concentration (mg/ml)	Albumin standard stock (µl)	Homogenization buffer (µl)
0.1	25	475
0.2	50	450
0.3	75	425
0.4	100	400
0.5	125	375

* Protein standards and albumin standard stock can kept in -20 ºC.

2.2 Alpha or Beta-naphthol standard (alpha or beta-naphthol stock concentration = 4 µg naphthol)

The alpha or beta-naphthol standard stock was prepared from 16 mg of alpha or beta-naphthol (Sigma) and 1 ml potassium phosphate buffer pH 7.0. Then 1 ml of alpha or beta-naphthol stock was diluted in 9 ml potassium phosphate buffer pH 7.2, producing a 10-fold dilution (32 µg naphthol), then a series of 0.5 ml volumes of 2-fold dilutions of alpha or beta-naphthol was prepared and diluted to 0.0625-2 µg naphthol, as follows;

Step	Amt Tranferred	Source	Homogenization buffer	Step dilution	Final conc. (µg naphthol)
1	250 µl	Stock (4 µg naphthol)	250 µl	1/2	2
2	250 µl	#1 (2 µg naphthol)	250 µl	1/2	1
3	250 µl	#2 (1 µg naphthol)	250 µl	1/2	0.5
4	250 µl	#3 (0.5 µg naphthol)	250 µl	1/2	0.25
5	250 µl	#4 (0.25 µg naphthol)	250 µl	1/2	0.125
6	250 µl	#5 (0.125 µg naphthol)	250 µl	1/2	0.0625

* After using, all of standard set will leave.

2.3 Cytochrome c standard (cytochrome c stock concentration = 0.1 µg/µl)

The cytochrome c standard stock was prepared from 10 mg of cytochrome c from horse heart bovine (Sigma) and 100 ml sodium acetate buffer pH 5.0. Then 100 µl of cytochrome c stock was divided in 1.5 ml microcentrifuge tube and kept at -20 ºC. After first thawing, 900 µl of sodium acetate buffer pH 5.0 was added into the microcentrifuge tube and diluted to 0.4-1.4 ng/µl concentration, as follows;

Concentration (ng/µl)	Cytochrome c stock (concentration 10 ng/µl) (µl)	Sodium acetate buffer pH 5.0 (µl)
0.4	40	960
0.6	60	940
0.8	80	920
1.0	100	900
1.2	120	880
1.4	140	860

* After using, all of standard set will leave.

2.4 *p*-nitrophenol standard (*p*-nitrophenol stock concentration = 8 µg *p*-nitrophenol)

The *p*-nitrophenol standard stock was prepared from 6.4 mg of *p*-nitrophenol (Sigma) and 1 ml homogenization buffer. Then a series of 0.5 ml volumes of 2-fold dilutions of *p*-nitrophenol was prepared and diluted to 0.0625-4 µg *p*-nitrophenol, as follows;

Step	Amt Tranferred	Source	Homogenization buffer	Step dilution	Final conc. (µg *p*-nitro phenol)
1	250 µl	Stock (8 µg *p*-nitrophenol)	250 µl	1/2	4
2	250 µl	#1 (4 µg *p*-nitrophenol)	250 µl	1/2	2
3	250 µl	#2 (2 µg *p*-nitrophenol)	250 µl	1/2	1
4	250 µl	#3 (1 µg *p*-nitrophenol)	250 µl	1/2	0.5
5	250 µl	#4 (0.5 µg *p*-nitrophenol)	250 µl	1/2	0.25
6	250 µl	#5 (0.25 µg *p*-nitrophenol)	250 µl	1/2	0.125
7	250 µl	#6 (0.125 µg *p*-nitrophenol)	250 µl	1/2	0.0625

* After using, all of standard set will leave.

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Lecture attendance on Nanotechnology and applications, Program in Chemistry, Faculty of Science, Maejo University, Chiang Mai, Thailand in 2013.

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Conference attendance on the Seminar "Analytical of Advanced chromatography technique and Ultracentrifugation of Sedimentation for Molecules" Chiangmai Grandview, Chiang Mai, Thailand in 2016.

Conference attendance on the Seminar on Asia Insect and Biomedical Research 2016. Chiangmai Grandview, Chiang Mai, Thailand in 2016.

Research assistant on the role of acetylcholinesterases in *Aedes aegypti* project, Research Institute for Health Sciences, Chiang Mai University, Thailand in 2018.

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