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Appendix I

2.1 Chemicals.

Chemicals	Product of company
Acetic acid	J.T. Baker Chemical Co
Acrylamide	Merck
ADP	Sigma
2,2'-azino-di(3-ethylbenzthiazoline) sulfonic acid(ABTS)	Zymed Laboratories, Inc
BSA	Sigma
Citric acid	BDH chemicals, Ltd.
Coomassie brilliant blue G-250	Fluka
Coomassie brilliant blue R-250	Sigma
3,3'-diaminobenzidine tetrahydrochloride(DAB)	Zymed Laboratories, Inc
Excellulose GF-5	Pierce
Ferric chloride($FeCl_3$)	Sigma
Freund's complete adjuvant(FCA)	Difco Laboratories, Ltd.
Freund's incomplete adjuvant(FIA)	Difco Laboratories, Ltd.
L-glutamine	Sigma
Glycine	Sigma
Horseradishperoxidase-goat anti-rabbit IgG(HRP)	Zymed Laboratories, Inc
Hydrogen peroxide(H_2O_2)	Fluka
Hydroxylamine hydrochloride (NH_2OH-HCl)	Fluka
Imidazole-HCl	Fluka
Immobilized Protein A	Pierce

Manganese sulfate ($MnSO_4$)	M & B laboratory
Methanol	J.T. Baker Chemical Co
N,N'-methylenebis acrylamide	Kodak
Phosphoric acid	Merck
Reactive Blue Sepharose CL-6B	Sigma
Sepharose-4B	Phamacia
Skim milk	Difco Laboratories, Ltd.
Sodium arsenate	BDH chemicals, Ltd.
Sodium dodecyl sulfate(SDS)	Bio-Rad
Sodium hydroxide(NaOH)	EKA Nobel Ltd.
Standard γ-glutamyl hydroxamate	Sigma
Standard molecular weight marker for SDS-PAGE	Sigma
TEMED	BDH chemicals, Ltd.
Thimerosal	Merck

Appendix II

Preparetion of buffers

0.1 M citric acid buffer pH 4.2

Citric acid	21.014	g/l
Na_2HPO_4	28.392	g/l

Mixed 24.0 ml Citric acid solution and 26.0 ml Na_2HPO_4 adjust pH to 4.2 and added the H_2O to 100 ml.

0.1 M phosphate buffer (PBS1)

Na_2HPO_4	14.19	g/l
NaH_2PO_4	13.79	g/l
NaCl	8.77	g/l
NaN_3	0.1	g/l

Na_2HPO_4 solution was mixed with NaH_2PO_4 solution and adjust pH to 7.0 then NaCl and NaN_3 were added.

0.1 M phosphate buffer(PBS2)(Johnstone and Thorpe, 1982)

NaCl	8.0	g/l
KCl	0.20	g/l
KH_2PO_4	0.20	g/l
Na_2HPO_4	1.15	g/l
Thimerosol	0.1	g/l

Adjust pH to 7.4 with 0.1 M HCl or 0.1 M NaOH.

100 mM tris-HCl buffer pH 7.6(TBEM)

Tris-HCl	12.114	g/l
EDTA	0.3722	g/l
2-mercaptoethanol	0.79	µl
$MgCl_2$	0.203	g/l

After dissolving of all those, the solution was adjust pH to 7.6 by 6 M HCl.

50 mM Tris-HCl pH 7.5 (TBS)

Tris-HCl	6.057	g/l
NaCl	8.766	g/l

Adjust pH to 7.5 with 6 M HCl.

Appendix III

Preparation of stock solution and gel for PAGE.

a. Stock 30 % acrylamide, 0.8 % bis-acrylamide was prepared and stored in a dark bottle at 4 °C.

b. Stock 10 % acrylamide, 2.5 % bis-acrylamied was prepared and stored in a dark bottle at 4 °C.

c. 0.5 M Tris-HCl, 0.46 % v/v TEMED, pH 6.7

d. 3.0 M Tris-HCl, 0.23 % v/v TEMED, pH 8.8

e. 0.004 % riboflavin

Working solutions for polyacrylamide gel electrophoresis(PAGE) were prepared as follow:

Resolving gel solution : 2 parts stock a, 1 part stock d, 1 part stock e and 4 parts distilled water

Stacking gel solution : 2 parts stock b, 1 part stock c, 1 part stock e and 4 part distilled water.

Electrode buffer : 6 g Tris, 28.8 g glycine and distilled water to 1 l pH 8.3 diluted 10 folds before use.

Sample preparation : Add 30 ul of 0.25 % bromophenol blue. with glycerol in sample solution 100 µl.

Preparation of stock solution and gel for SDS-PAGE.

Working solution for SDS denaturing gel electrophoresis(SDS-PAGE) were prepared as follows:

Resolving gel:

distilled water	8.2 ml
1.5 M Tris HCl, 0.45 % SDS pH 8.8	5.0 ml

30 % acrylamide, 0.8 % bisacrylamide	6.7 ml
TEMED	10 µl
10 % Ammonium persulfate	100 µl

Stacking gel:

distilled water	7.0 ml
0.5 M Tris HCl, 0.4 % SDS PH 6.7	3.0 ml
30 % acrylamide, 0.8 % bisacrylamide	1.8 ml
TEMED	15 µl
10 % Ammonium persulfate	150 µl

Electrode buffer : 15.1 g Tris, 72.04 g glycine, 5 g and distilled water to 500 ml, pH 8.3 diluted 10 folds before use.

Sample preparation : Sample solutions were prepared in microfuge tube by adding an equal volume of 0.5 M Tris-HCl buffer pH 6.7 containing 0.4 % SDS , 5 % 2-mercaptoethanol, 0.002 % Bromophenol blue, 10 % glycerol. The sample was heated for 5 min in a boiling water bath. After cooling, the solution were centrifuged and the supernatant were transferred to other tubes before loading.

Protein Staining solution : Coomassie brilliant blue R 0.25 % methanol 45 % and acetic acid 9 %.

Destaining solution: Acetic acid 7 % and methanol 25 %

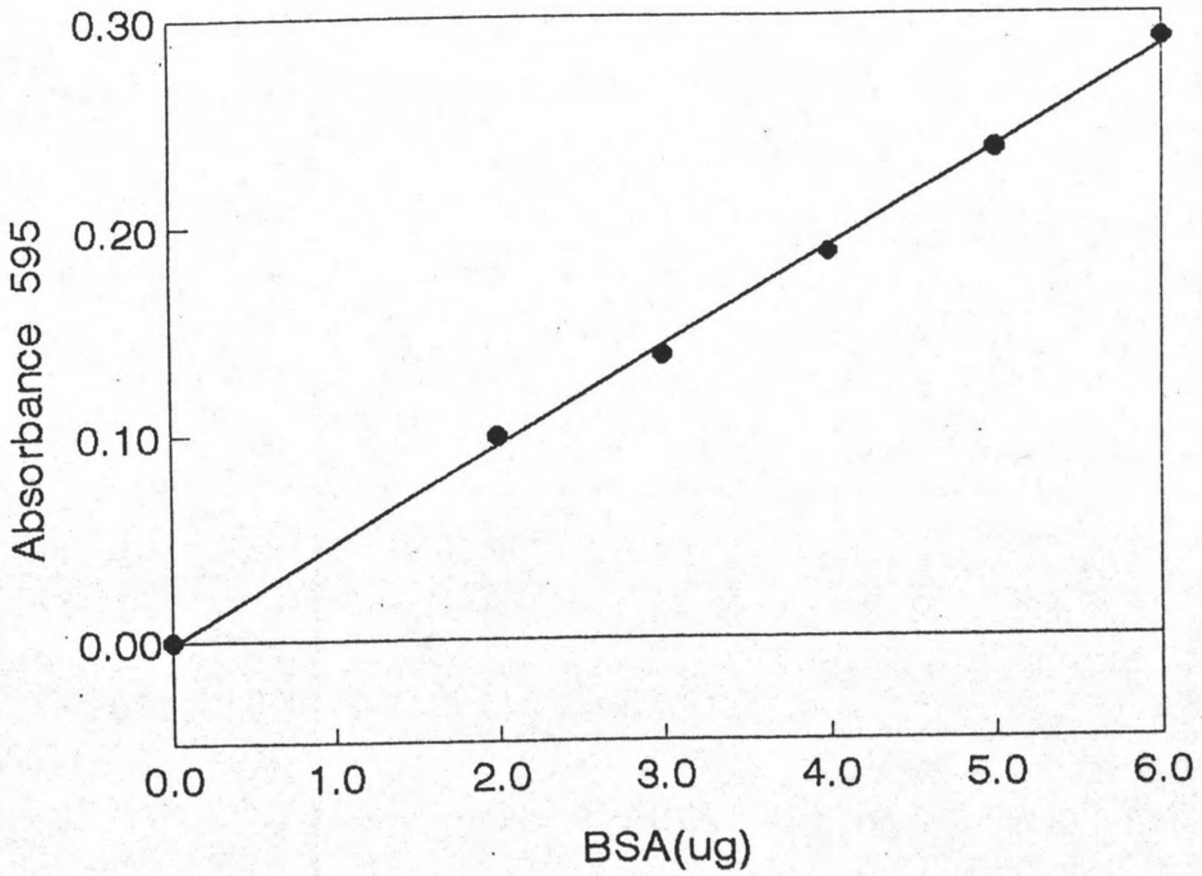
Preparation of protein reagent (Bradford's method)

Coomassie brilliant blue G 100 mg was dissolved in 50 ml 95 % ethanol. This solution was mixed with 100 ml of 85 % w/v phosphoric acid, and then diluted with distilled water to the final volume of 1 l and filtered.

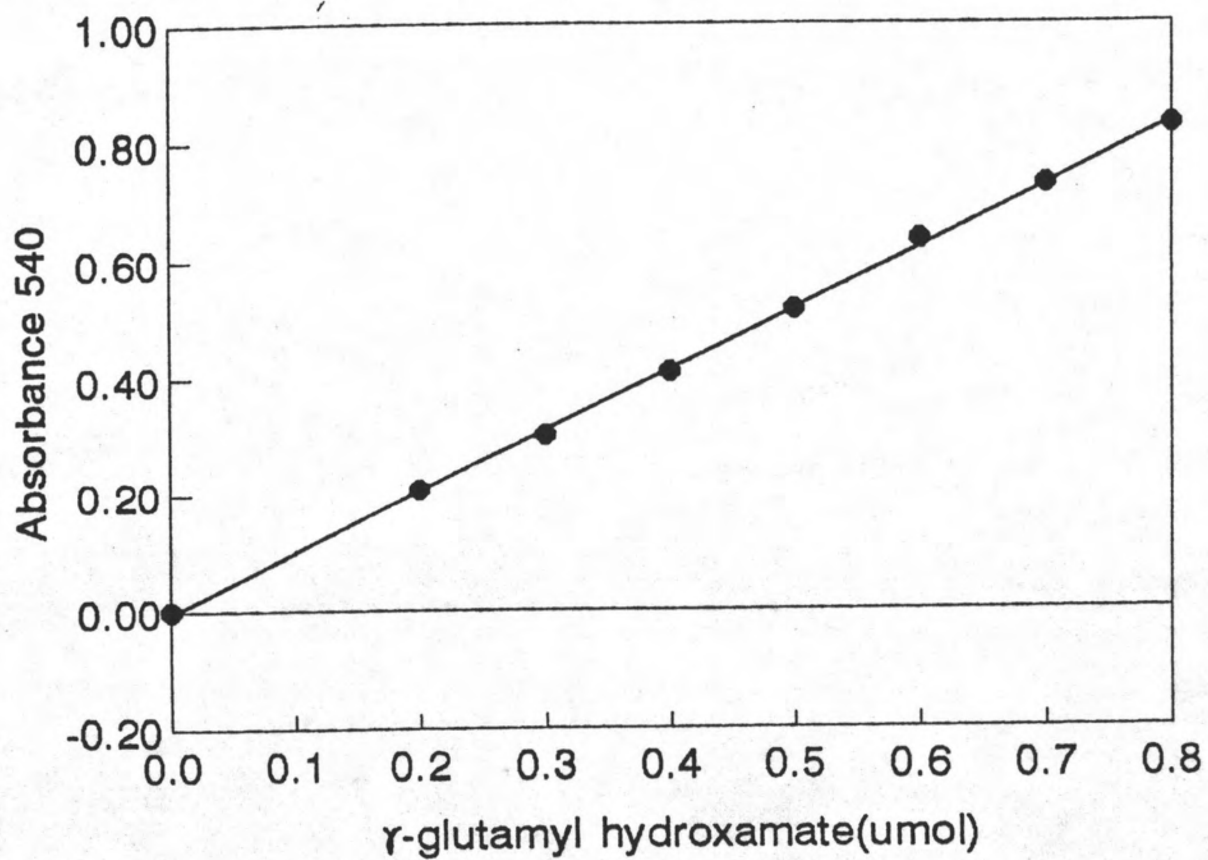
This reagent 1.0 ml was added to 100 µl of sample solution and mixed either by vortexing or several inversion and measured for

absorbanceat 595 nm after 2-15 min. Standard protein solutions(BSA) in every assay.

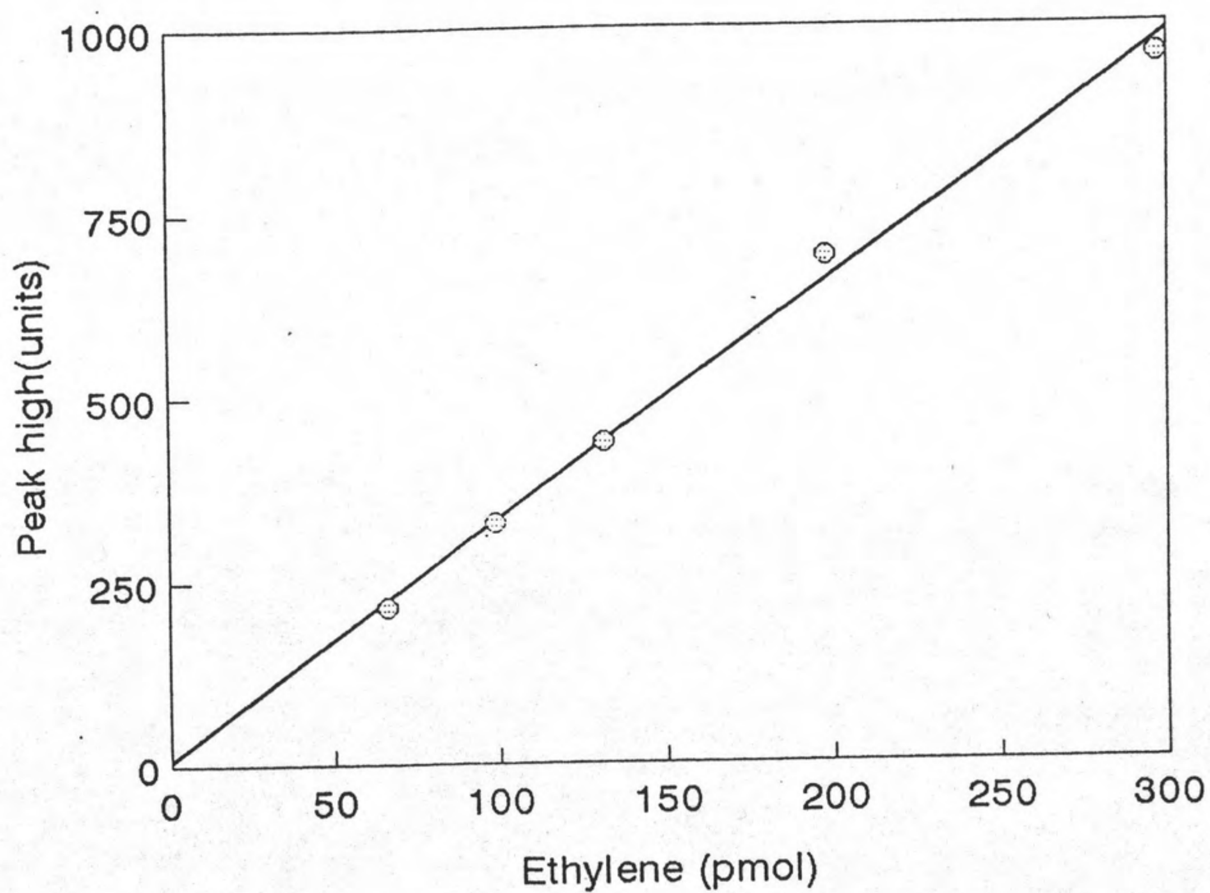
APPENDIX IV



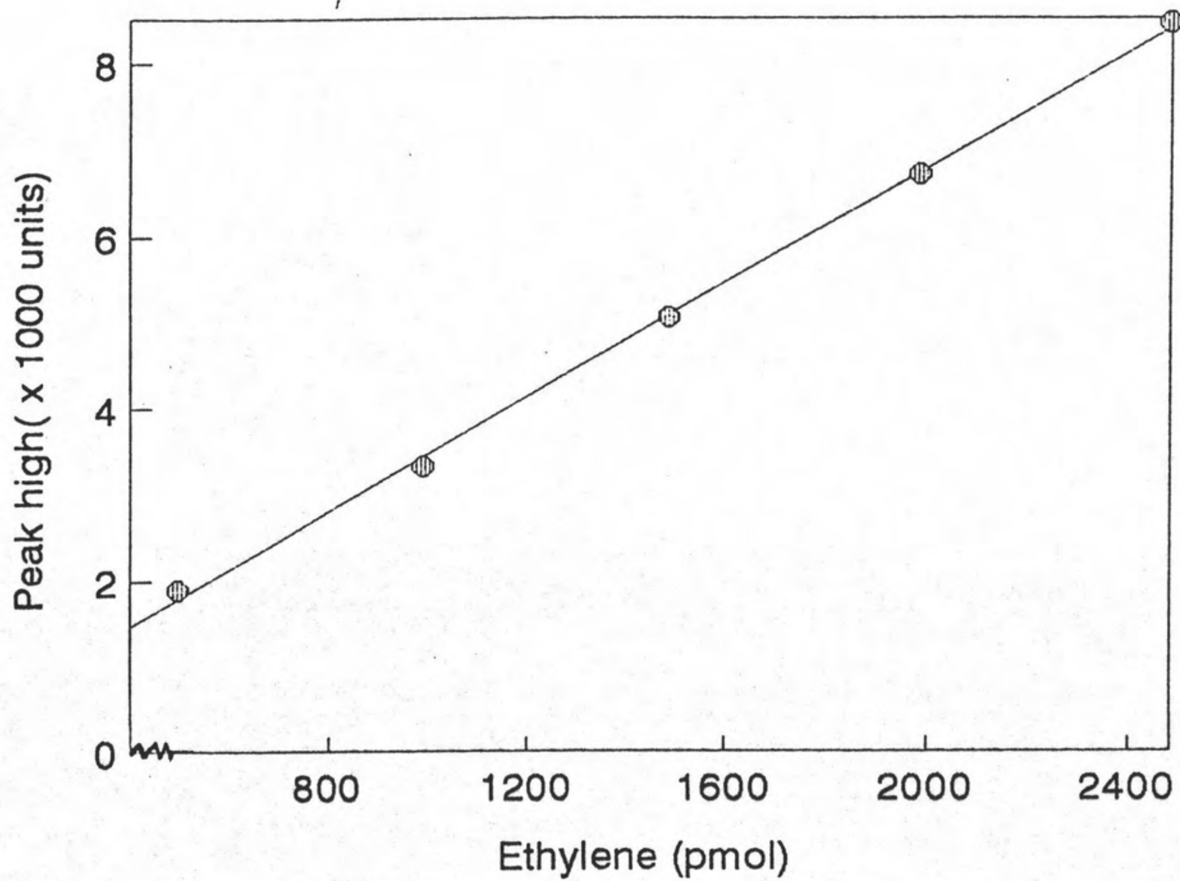
Standard curve of BSA.



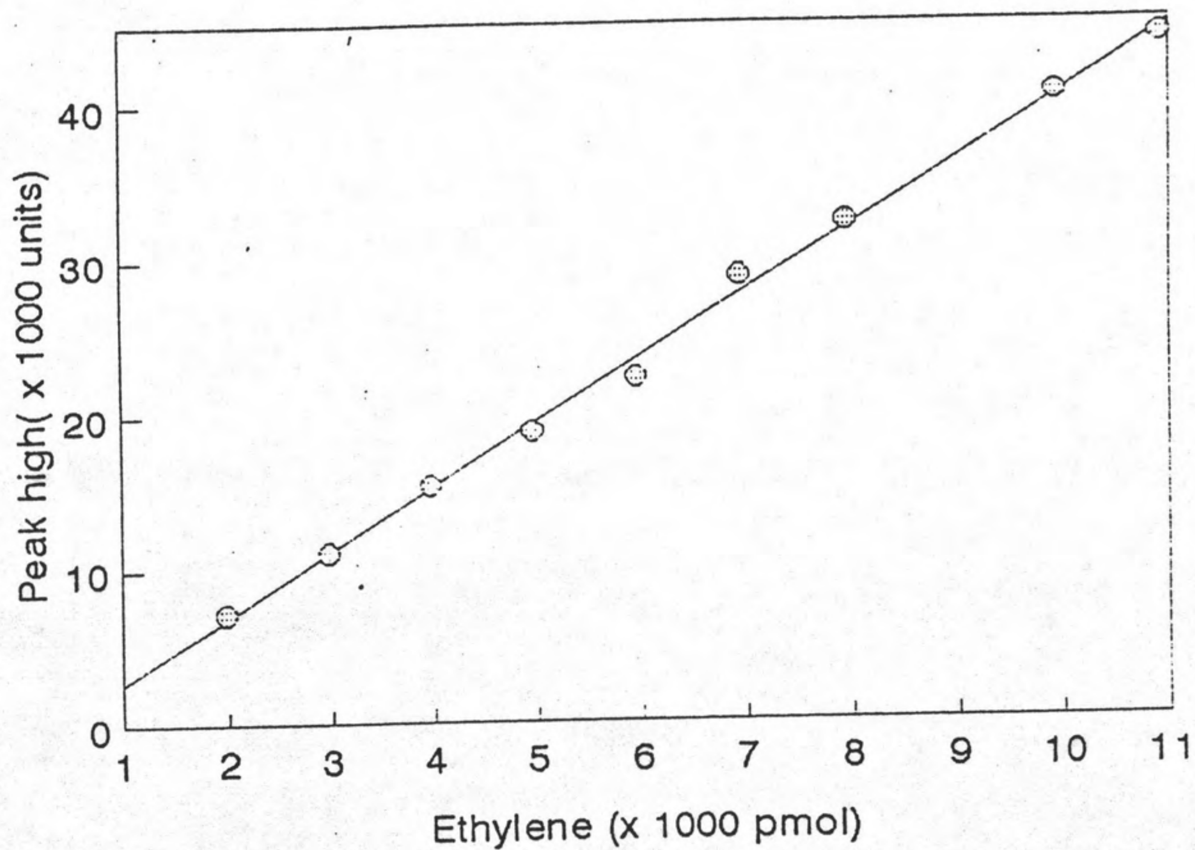
Standard curve of γ-glutamyl hydroxamate



Standard curve of ethylene ranging 60-300 pmol.



Standard curve of ethylene ranging 500-2500 pmol.



Standard curve of ethylene ranging 2000-11000 pmol.

BIOGRAPHY

Miss Ladda Saengduan was born on June 10, 1963 in Nonthaburee Thailand. She graduated with the Bachelor degree of Science in Biochemistry from the Faculty of Sience Chulalongkorn University in 1990.