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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



APPENDICES

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Appendix 1: Preparation for polyacrylamide gel electrophoresis

1) Stock reagents

30% Acrylamide, 0.8% bis-acrylamide, 100 ml

acrylamide	29.2 g
N,N'-methylene-bis-acrylamide	0.8 g

Adjusted volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris(hydroxymethyl)-aminomethane	18.17 g
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Adjusted pH to 8.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

2 M Tris-HCl pH 8.8

Tris(hydroxymethyl)-aminomethane	24.2 g
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Adjusted pH to 8.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris(hydroxymethyl)-aminomethane	6.06 g
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Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

1 M Tris-HCl pH 6.8

Tris(hydroxymethyl)-aminomethane	12.1 g
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Adjusted pH to 6.8 with 1 M HCl and adjusted volume to 100 ml with distilled water

Solution B (SDS-PAGE)

2 M Tris-HCl pH 8.8	75 ml
10% SDS	4 ml
distilled water	21 ml

Solution C (SDS-PAGE)

1 M Tris-HCl pH 6.8	50 ml
10% SDS	4 ml
distilled water	46 ml

2) Non-denaturing PAGE**7.5% Separating gel**

30% acrylamide solution	2.5 ml
1.5 M Tris-HCl pH 8.8	2.5 ml
distilled water	5.0 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50 μl
TEMED	10 μl

5.0% stacking gel

30% acrylamide solution	0.67 ml
0.5 M Tris-HCl pH 6.8	1.0 ml
distilled water	2.3 ml
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	30 μl
TEMED	5 μl

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Appendix 2: Preparation of Periodic Acid-Schiff staining solution

Fixative solution

Trichloroacetic acid	12.5 g
Distilled water	100 ml

Schiff's reagent

1. Dissolved 1 g of Basic Fuchsin 200 ml of boiling distilled water. Stirred for 5 minutes and cooled to 50°C.
2. Filtered and added the filtrate to 20 ml of 1 N HCl.
3. Cooled to 25°C and added 1 g of sodium or potassium metabisulfite (Fresh metabisulfite was required for optimum result).
4. Left this solution in dark for 12-24 hours.
5. Added 2 g of activated charcoal, shaken for 1 minute, filtered and stored at room temperature.

1% Periodic acid in 3% Acetic acid

Periodic acid	1 g
dissolved in 3% acetic acid	100 ml

0.5% Sodium Metabisulfite

Sodium metabisulfite	0.5 g
Distilled water	100 ml

7% Acetic acid

Glacial acetic acid	7 ml
Distilled water	93 ml

Appendix 3: Preparation for isoelectric focusing gel electrophoresis

Monomer-ampholyte solution

30% Acrylamide solution	0.9 ml
1.0% Bis-acrylamide solution	1.25 ml
Ampholyte pH 5-7	0.243 ml
Distilled water	1.39 ml
50% Sucrose	1.186 ml
TEMED	2 μ l
0.02 M $(\text{NH}_4)_2\text{S}_2\text{O}_8$	39 μ l

Fixative solution, 100 ml

Sulfosalicylic acid	4 ml
Trichloroacetic acid	12.5 ml
Methanol	30 ml

Immerse gels in this solution for 30 minutes.

Staining solution, 100 ml

Ethanol	27 ml
Acetic acid	10 ml
Coomassie brilliant blue R-250	0.04 ml
CuSO_4	0.5 ml
Distilled water	63 ml

Dissolve the CuSO_4 in water before adding the alcohol. Either dissolve the dye in alcohol or add it to the solution at the end.

Immerse the gel in stain for approximately 1-2 hours.

Appendix 4: Preparation for buffer solution

- 0.2 M Potassium Acetate pH 3.0, 4.0 and 5.0

CH₃COOK 1.96 g

Adjusted to pH 3, 4 or 5 by 0.2 M acetic acid and adjusted volume to 100 ml with distilled water.

- 0.2 M Phosphate pH 6.0

KH₂PO₄ 2.27 g

K₂HPO₄ 0.58 g

distilled water 100 ml

- 0.2 M Phosphate pH 7.0

KH₂PO₄ 0.91 g

K₂HPO₄ 2.32 g

distilled water 100 ml

- 0.2 M Tris-HCl pH 8.0 and 9.0

Tris(hydroxymethyl)-aminometane 2.42 g

Adjusted to pH 8.0 or 9.0 by 1 M HCl and adjusted to 100 ml with distilled water.

- 0.2 M Tris-Glycine NaOH pH 10.0 and 11.0

Glycine 1.5 g

Adjusted to pH 10.0 or 11.0 by 1 M NaOH and adjusted volume to 1000 ml with distilled water.

Appendix 5 Reactivities of amino acid side chains

Reagent	-NH ₂	-SH	-NH ₂	-SH	-S-S-	-S-CH ₃
Acetic anhydride	+++	+++ ^b	+++ ^c	+++ ^c	+++ ^b	-
<i>N</i> -Acetylimidazole	+++	+++ ^b	+++ ^c	+++ ^b	-	-
Acrylonitrile	+++	+++	-	-	-	-
Aldehyde/NaBH ₄	+++	-	-	-	-	-
<i>N</i> -Bromosuccinimide	-	+++	++	+	-	+++
<i>N</i> -carboxyanhydrides	+++	-	-	-	-	-
Cyanate	+++	+++ ^b	++ ^b	+ ^b	-	+ ^b
Cyanogen bromide	-	+	-	-	-	-
1,2-cyclohexanedione	+	-	-	-	+++	-
Diacetyl trimer	+	-	-	-	+++	-
Diazoacetates	-	++	-	-	-	+++
Diazonium salts	+++	+	+++	+++	+	-
Diethylpyrocarbonate	+++	-	-	+++ ^c	-	-
Diketone	+++ ^c	-	+	-	-	-
Dinitrofluorobenzene	+++	+++	++	++	-	-
5,5'-dithiobis(2-nitrobenzoic acid)	-	+++ ^c	-	-	-	-

Appendix 5 Reactivities of amino acid side chains (continued)

Reagent	-NH ₂	-SH	-NH ₂	-SH	-NH ₂	-SH	-NH ₂	-SH	-S-S-	-S-CH ₃
Ethyleneimide	-	+++	-	-	-	-	-	-	-	-
<i>N</i> -ethylmaleimide	++	+++	-	-	-	-	-	-	-	-
Ethyl thiotrifluoroacetate	+++ ^b	-	-	-	-	-	-	-	-	-
Formaldehyde	+++	+++	+++	+++	+	-	+	-	-	
Glyoxal	++	-	-	-	+++	-	-	-	-	
Haloacetates	+	+++	-	+	-	-	-	-	+	
Hydrogen peroxide	-	+++	-	-	-	-	+	+	+++	
2-hydroxy-5-nitrobenzyl bromide	-	++	-	-	-	-	+++	-	-	
Iodine	-	+++	+++	+++	-	-	--	-	-	
<i>O</i> -iodosobenzoate	-	+++	-	-	-	-	-	-	-	
Maleic anhydride	+++ ^c	++ ^c	++ ^b	++ ^b	-	-	-	-	-	
<i>p</i> -mercuribenzoate	-	+++	-	-	-	-	-	-	-	
Methanol/HCl	-	-	-	-	+++	-	-	-	-	
2-methoxyl-5-nitropropone	+++ ^c	-	-	-	-	-	-	-	-	
Methyl acetimidate	+++	-	-	-	-	-	-	-	-	
<i>O</i> -methylisourea	+++	-	-	-	-	-	-	-	-	
Nitrous acid	+++	+++	±	-	-	-	-	+	-	

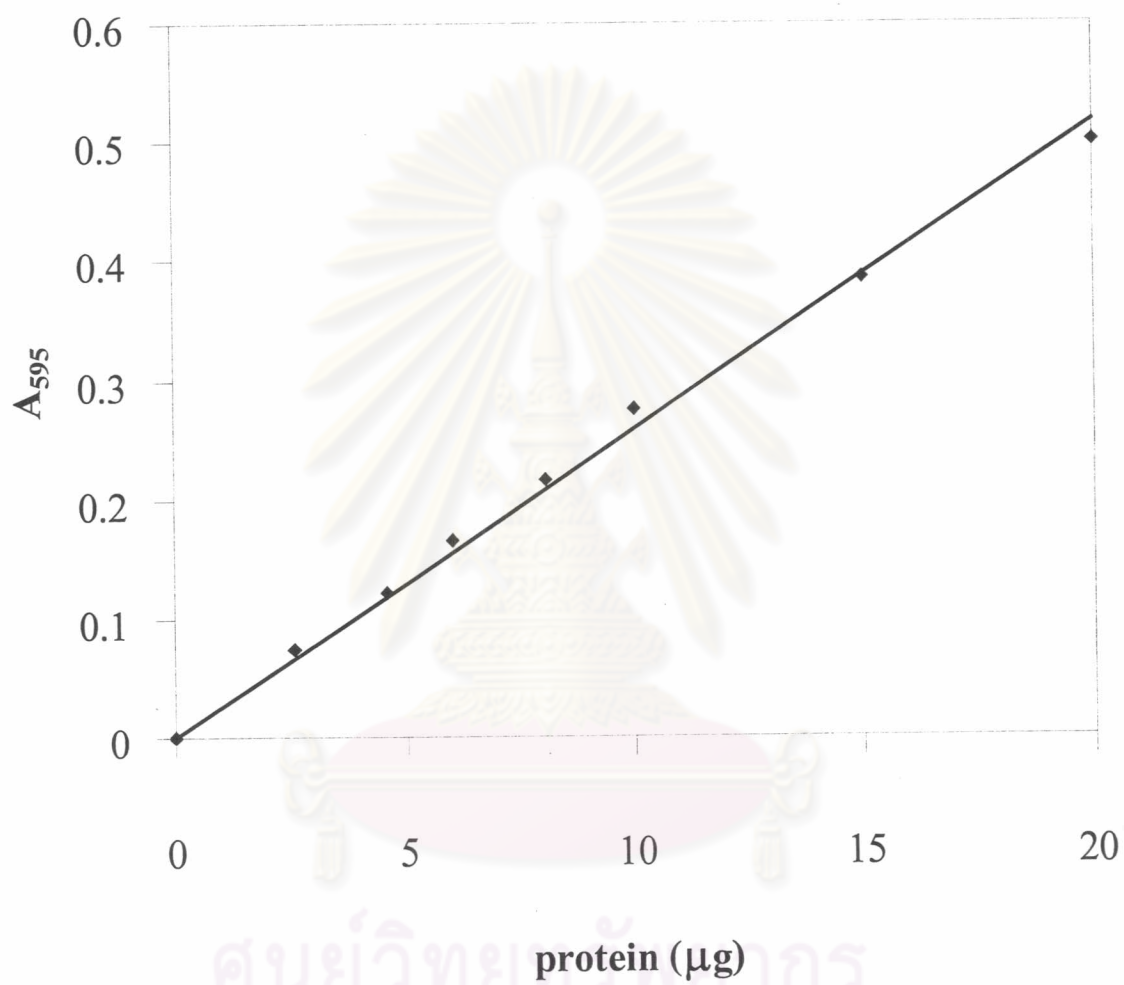
Appendix 5 Reactivities of amino acid side chains (continued)

Reagent	-NH ₂	-SH	-NH	-NH ₂	-SH	-S-S-	-S-CH ₃		
Performic acid	-	+++	-	-	-	++	+++	+++	
Phenylglyoxal	++	-	-	-	+++	-	-	-	
Photooxidation	-	+++	<u>+++</u>	+++	-	-	+++	<u>±</u>	+++
Sodium borohydride	-	+++ ^b	++ ^b	++ ^b	-	-	-	-	-
Succinic anhydride	+++	+++	-	-	-	-	+++	-	-
Sulfite	-	+++	+++	+++	-	-	-	-	-
Sulfonyl halides	+++	+++	+++	-	-	-	+	-	+
Tetranitromethane	-	+++	+++	-	-	-	+	-	-
Tetrathionate	-	+++	-	-	-	-	-	+++	-
Thiols	-	-	-	-	-	-	-	+++	-
Trinitrobenzenesulfonic acid	+++	++ ^b	-	-	-	-	-	-	-
Water-soluble carbodiimide and nucleophile	<u>±</u>	<u>±</u>	<u>±</u>	-	+++	-	-	-	-

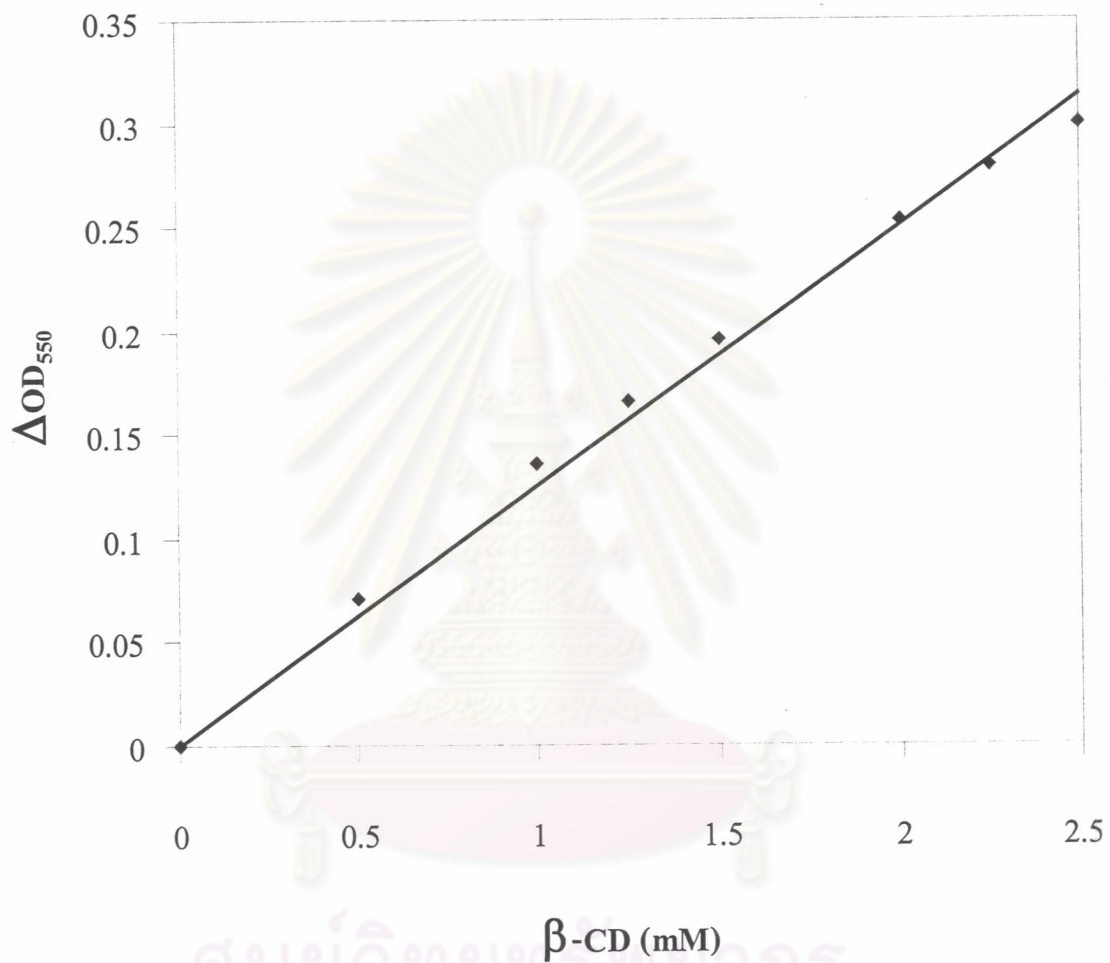
^a -, +, ++, and +++ indicate relative reactivities; ±, +++, and +++ likewise indicate relative reactivities which may or not be attained depending on the condition used.

^b Spontaneously reversible under the reaction conditions or upon dilution, regenerating original group.

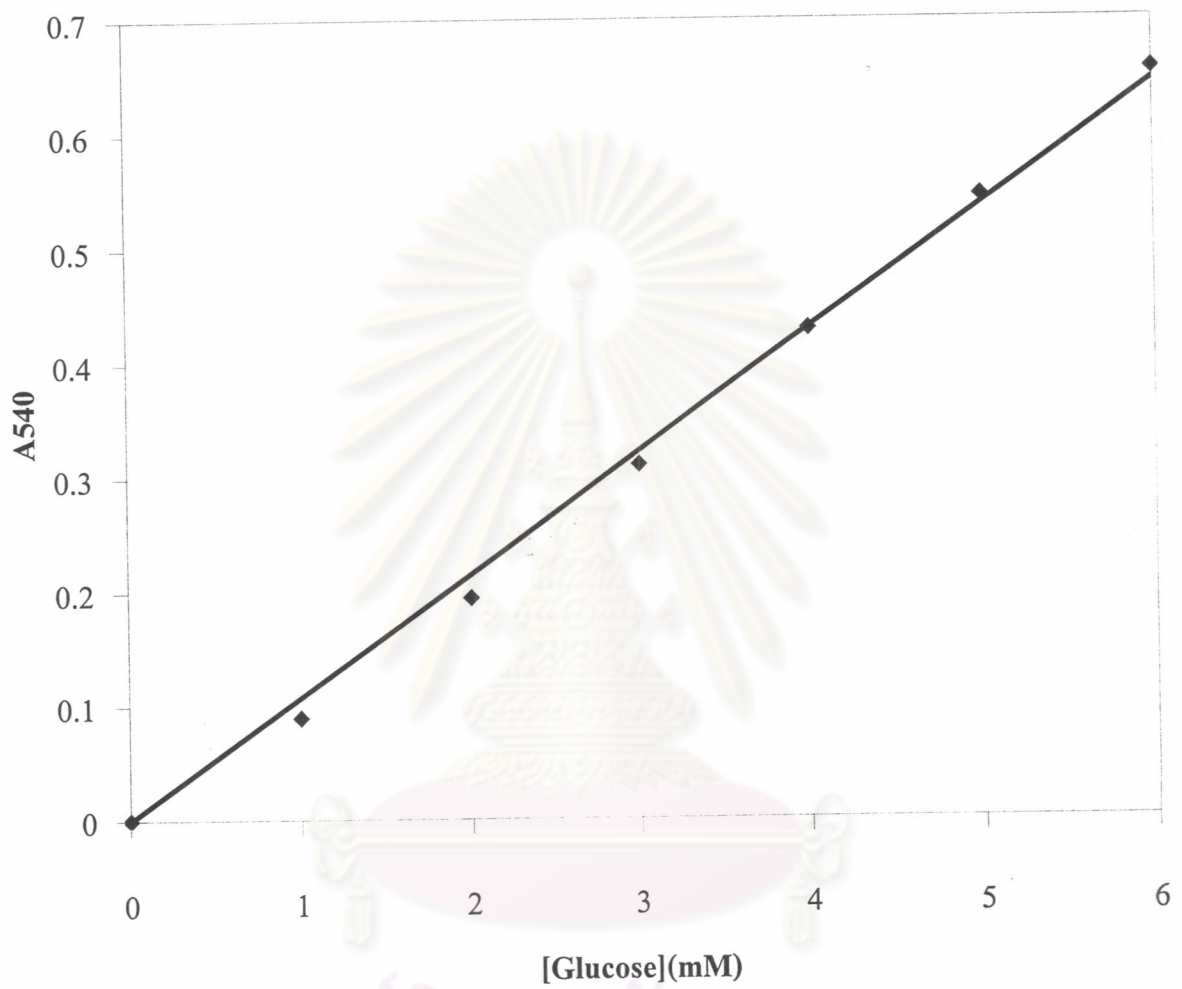
^c Easily reversible, regenerating original group.

Appendix 6: Standard curve for protein determination by Bradford's method

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Appendix 7: Standard curve of β -cyclodextrin by phenolphthalein method

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Appendix 8: Standard curve of glucose by dinitrosalicylic acid method

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BIOGRAPHY

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