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APPENDICES

APPENDIX A : Prepration of stock solutions for enzymatic assays**135 mM Calcium chloride (CaCl₂)**

Calcim chloride 0.749 g

Adjust volume to 50 ml with distilled water.

50 mM Potassium cyanide (KCN)

Potassium cyanide 0.163 g

Adjust volume to 50 ml with distilled water.

6 mM Phenazine methosulfate (PMS)

Phenazine methosulfate 0.092 g

Adjust volume to 50 ml with distilled water.

6.6 mM 2,6-Dichlorophenol-indophenol sodium salt dehydrate (DCIP)

2,6-Dichlorophenol-indophenol sodium salt dehydrate 0.108 g

Adjust volume to 50 ml with distilled water.

200 mM Polyethylene glycol 2000

Polyethylene glycol 2000 4 g

Adjust volume to 10 ml with distilled water.

200 mM Polyethylene glycol 4000

Polyethylene glycol 4000 8 g

Adjust volume to 10 ml with distilled water.

200 mM Polypropylene glycol 1000

Polypropylene glycol 2000 1.98 ml

Adjust volume to 10 ml with distilled water.

200 mM Polypropylene glycol 2000

Polypropylene glycol 2000 3.96 ml

Adjust volume to 10 ml with distilled water.

0.1 M Tris-HCl pH 8.0

Tris (hydroxymethyl) aminomethane 1.21 g

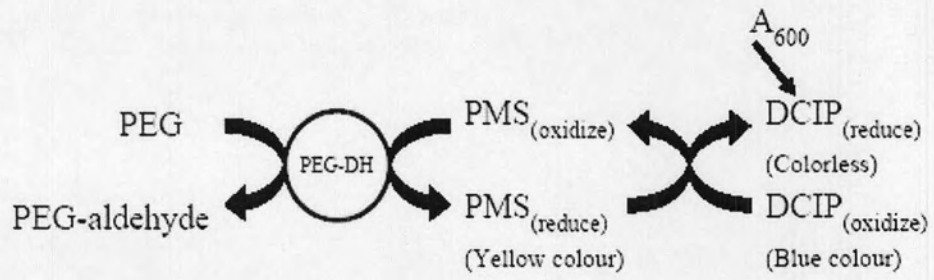
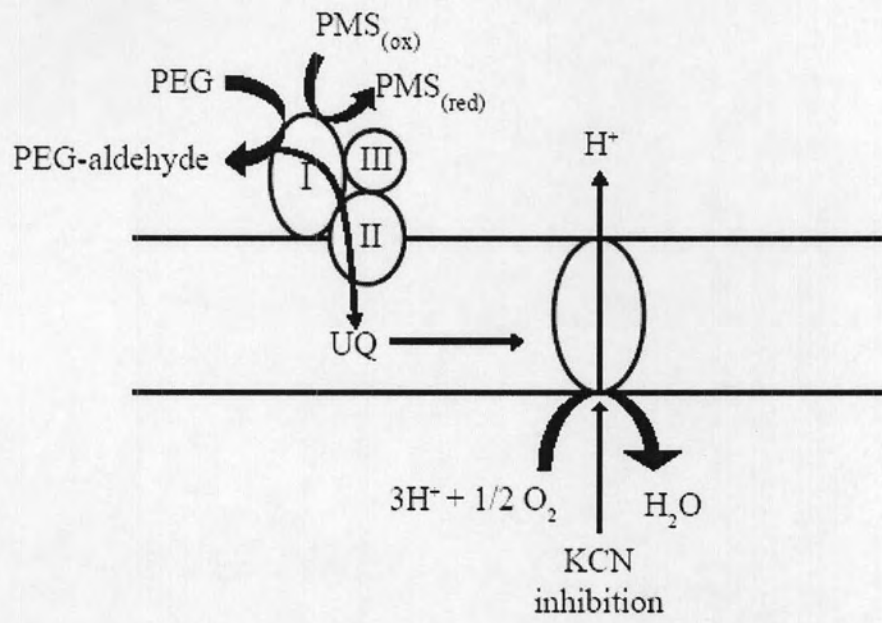
Adjust pH to 8.0 with 1 M HCl and adjust volume to 100 ml with distilled water.

The reaction mixture consisted of

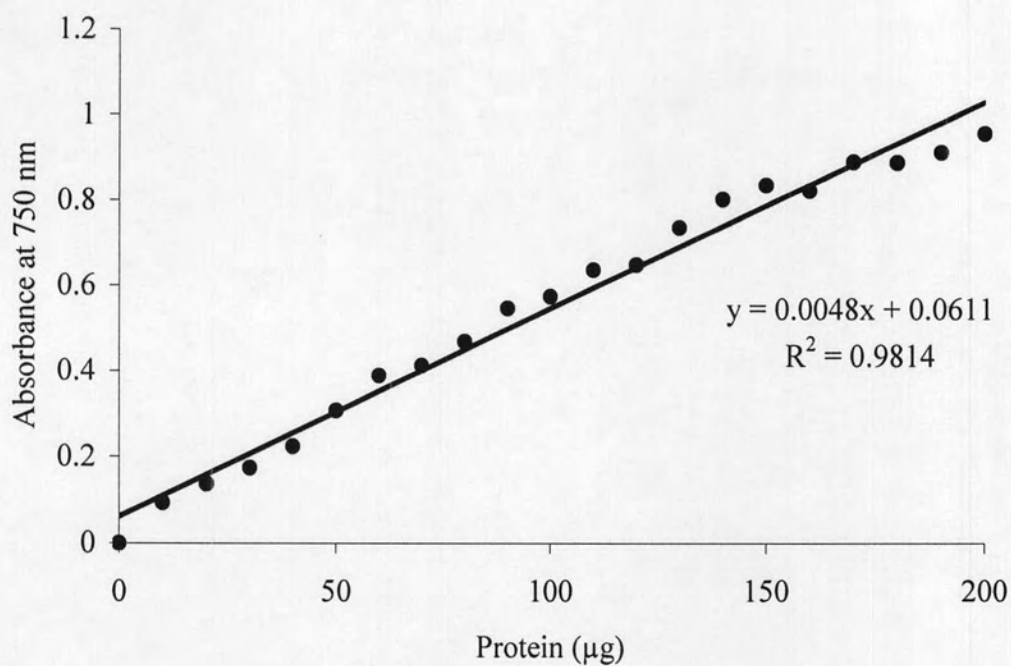
135 mM CaCl ₂	15	μl
50 mM KCN	20	μl
6 mM PMS	17	μl
6.6 mM DCIP	15	μl
200 mM PEG or PPG	50	μl
Enzyme	50	μl
1.0 Tris-HCl pH 8.0	833	μl

Total volume 1 ml

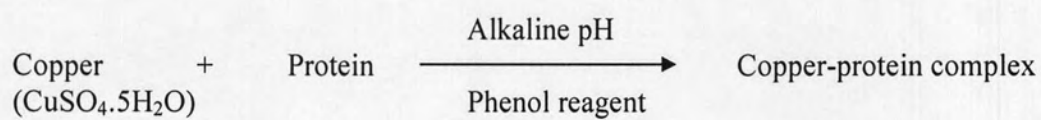
Principle : PMS-DCIP assay (Singer, 1974; Kawai, 1987)



APPENDIX B : Standard curve for protein determination by modified Lowry method



Principle : (Lowry *et al.*, 1951)



APPENDIX C : Preparation for protein determination**Reagent for determination of protein concentration****(modified from Lowry *et al.*, 1951)****Solution A: 2% sodium carbonate in 0.1 M sodium hydroxide
containing 0.5% sodium lauryl sulphate (SDS)**

Sodium carbonate	20	g
Sodium hydroxide	4	g
sodium lauryl sulphate	5	g

Dissolved in distilled water to 1 litre.

Solution B: 0.5% copper sulfate in 1% potassium sodium tartrate

Copper sulfate	1	g
Potassium sodium tartrate	2	g

Dissolved in distilled water to 200 ml.

Solution C: Phenol reagent (Folin-Ciocalteu's reagent)

Folin-Ciocalteu's reagent used in this work was reagent grade
from Carlo Erba Reagenti, France.

APPENDIX D : Preparation for polyacrylamide gel electrophoresis**1. Stock reagents****30% Acrylamide, 0.8% bis-acrylamide, 100 ml**

Acrylamide 29.2 g

N,N'-methyl-bis-acrylamide 0.8 g

Adjust volume to 100 ml with distilled water.

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl) aminomethane 18.17 g

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

2.0 M Tris-HCl pH 8.8

Tris (hydroxymethyl) aminomethane 24.2 g

Adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl) aminomethane 6.06 g

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

1.0 M Tris-HCl pH 6.8

Tris (hydroxymethyl) aminomethane 12.1 g

Adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled water.

2. Stock reagents for SDS-PAGE**Solution B**

2.0 M Tris-HCl pH 8.8	75	ml
10% SDS	4	ml
Distilled water	21	ml

Solution C

1.0 M Tris-HCl pH 6.8	50	ml
10% SDS	4	ml
Distilled water	46	ml

3. Non-denaturing PAGE**Calculation for X% separating gel**

30% Acrylamide solution	X/3	ml
1.5 M Tris-HCl pH 8.8	2.5	ml
Distilled water	(7.5-X/3)	ml
10% Ammonium persulfate	50	μl
TEMED	5	μl (10 μl if X<8%)

Total volume	10	ml
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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% Ammonium persulfate	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% Ammonium persulfate	30	μl
TEMED	5	μl

5X Sample buffer

1.0 M Tris-HCl pH 6.8	3.1	ml
Glycerol	5.0	ml
1% Bromophenol blue	0.5	ml
Distilled water	1.4	ml

One part of sample buffer was added to five parts of sample.

Electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl) aminomethane	3.03	g
Glycine	14.40	g

Dissolve in distilled water to 1 litre. Do not adjust pH with acid or base

(final pH should be 8.3).

4. SDS-PAGE**Calculation for X% separating gel**

30% Acrylamide solution	X/3	ml
Solution B	2.5	ml
Distilled water	(7.5-X/3)	ml
10% Ammonium persulfate	50	μl
TEMED	5	μl (10 μl if X<8%)

Total volume	10	ml
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10% Separating gel

30% Acrylamide solution	3.33	ml
Solution B	2.5	ml
Distilled water	4.17	ml
10% Ammonium persulfate	50	μl
TEMED	5	μl

5.0% Stacking gel

30% Acrylamide solution	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% Ammonium persulfate	30	μl
TEMED	5	μl

5X Sample buffer

1.0 M Tris-HCl pH 6.8	0.6	ml
50% Glycerol	5.0	ml
10% SDS	2.0	ml
2-Mercaptoethanol	0.5	ml
1% Bromophenol blue	1.0	ml
Distilled water	0.9	ml

One part of sample buffer was added to five parts of sample. The mixture was heated 5 min. in boiling water before loading to the gel.

Electrophoresis buffer, 1 litre

(25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl) aminomethane	3.03	g
Glycine	14.40	g
SDS	1.0	g

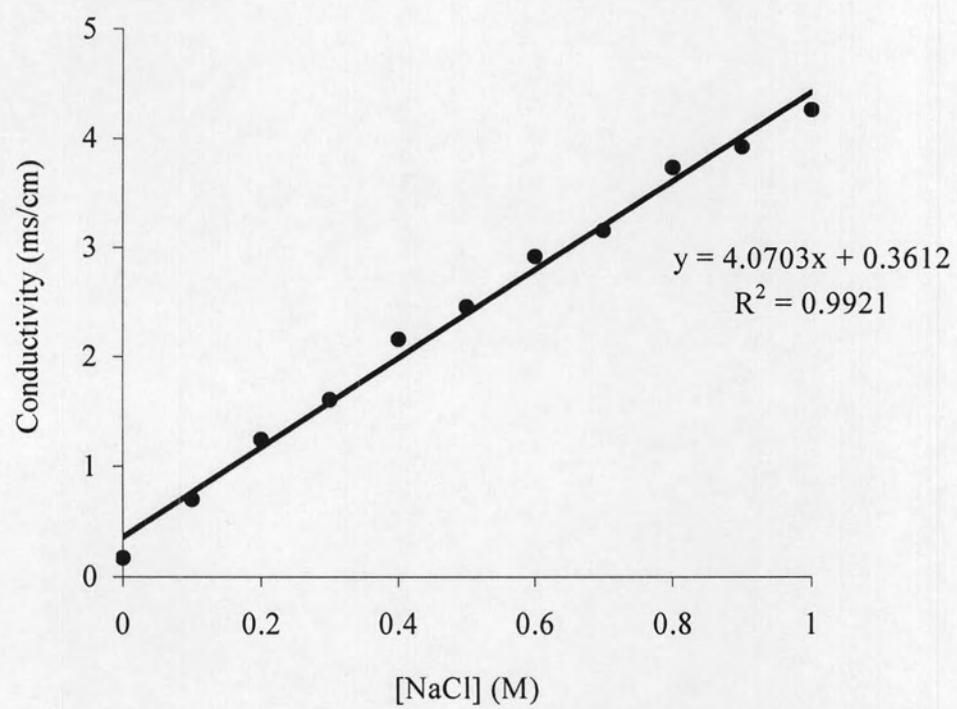
Dissolve in distilled water to 1 litre. Do not adjust pH with acid or base (final pH should be 8.3).

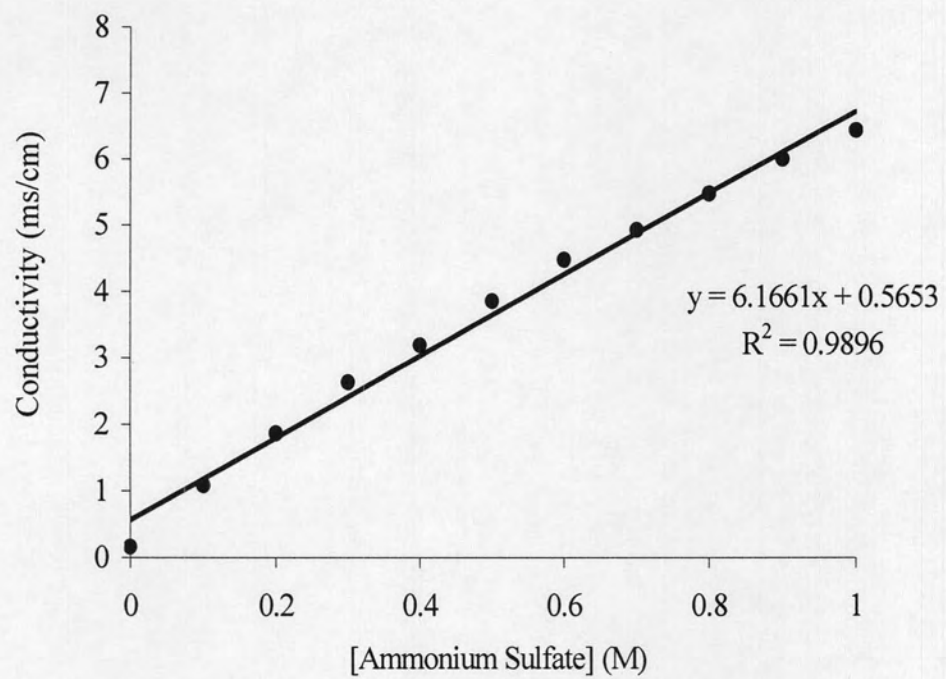
5. Staining solution, 1 litre

Coomassie brilliant blue R-250	1.0	g
Methanol	450	ml
Distilled water	450	ml
Glacial acetic acid	100	ml

6. Destaining solution, 1 litre

Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

APPENDIX E : Standard curve for conductivity of sodium chloride

APPENDIX F : Standard curve for conductivity of ammonium sulfate

APPENDIX G : Molecular weight determination of native PEG dehydrogenase and PPG dehydrogenase by polyacrylamide gel electrophoresis (PAGE)

4% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.9	6.0	4.6	4.0	0.7	0.7	66.7	66.7	182.4	182.4	182.4	0
Bovine serum albumin (66 kDa)	6.9	6.0	6.9	6.0	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
Ovalbumin (45 kDa)	6.9	6.0	6.9	6.0	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
Trypsin inhibitor type (21 kDa)	6.9	6.0	6.9	6.0	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	6.9	6.0	6.9	6.0	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PPG-DH	6.9	6.0	6.9	6.0	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0

5% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.7	7.5	4.3	4.7	0.6	0.6	64.2	62.7	180.7	179.7	180.2	0.7
Bovine serum albumin (66 kDa)	6.7	7.5	6.5	7.3	1.0	1.0	97.0	97.3	198.7	198.8	198.8	0.1
Ovalbumin (45 kDa)	6.7	7.4	6.6	7.3	1.0	1.0	98.5	98.6	199.3	199.4	199.4	0
Trypsin inhibitor type (21 kDa)	6.7	7.4	6.7	7.3	1.0	1.0	100.0	98.6	200.0	199.4	199.7	0.4
PEG-DH	6.7	7.4	6.7	7.4	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PPG-DH	6.7	7.4	6.7	7.4	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0

6% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.0	6.7	3.1	3.4	0.5	0.5	51.7	50.7	171.3	170.5	170.9	0.6
Bovine serum albumin (66 kDa)	6.0	6.7	5.6	6.2	0.9	0.9	93.3	92.5	197.0	196.6	196.8	0.3
Ovalbumin (45 kDa)	6.0	6.7	5.8	6.4	1.0	1.0	96.7	95.5	198.5	198.0	198.3	0.4
Trypsin inhibitor type (21 kDa)	6.0	6.7	6	6.7	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	6.0	6.7	5.3	5.9	0.9	0.9	88.3	88.1	194.6	194.5	194.6	0.1
PPG-DH	6.0	6.7	5.9	6.6	1.0	1.0	98.3	98.5	199.3	199.3	199.3	0.1

7% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.0	6.8	2.5	3	0.4	0.4	41.7	44.1	162.0	164.5	163.2	1.8
Bovine serum albumin (66 kDa)	6.0	6.8	4.8	5.4	0.8	0.8	80.0	79.4	190.3	190.0	190.2	0.2
Ovalbumin (45 kDa)	6.0	6.8	5.1	5.8	0.8	0.9	85.0	85.3	192.9	193.1	193.0	0.1
Trypsin inhibitor type (21 kDa)	6.0	6.8	6	6.8	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	6.0	6.8	4.4	5.0	0.7	0.7	73.3	73.5	186.5	186.6	186.6	0.1
PPG-DH	6.0	6.8	5.2	5.9	0.9	0.9	86.7	86.8	193.8	193.8	193.8	0

8% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	7.0	6.8	2.8	2.4	0.4	0.4	40.0	35.3	160.2	154.8	157.5	3.8
Bovine serum albumin (66 kDa)	7.0	6.8	5.1	4.2	0.7	0.6	72.9	61.8	186.2	179.1	182.7	5.1
Ovalbumin (45 kDa)	7.0	6.8	5.6	5	0.8	0.7	80.0	73.5	190.3	186.6	188.5	2.6
Trypsin inhibitor type (21 kDa)	7.0	6.8	7	6.8	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	7.0	6.8	4.7	4.6	0.7	0.7	67.1	67.6	182.7	183.0	182.9	0.2
PPG-DH	7.0	6.8	4.0	3.9	0.6	0.6	57.1	57.4	175.7	175.9	175.8	0.1

9% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.9	6.4	2.8	2.2	0.4	0.3	40.6	34.4	160.8	153.6	157.2	5.1
Bovine serum albumin (66 kDa)	6.9	6.4	4.8	4.1	0.7	0.6	69.6	64.1	184.2	180.7	182.4	2.5
Ovalbumin (45 kDa)	6.9	6.4	5.1	4.7	0.7	0.7	74.0	73.4	186.9	186.6	186.7	0.2
Trypsin inhibitor type (21 kDa)	6.9	6.4	6.9	6.4	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	6.9	6.4	4.4	4.0	0.6	0.6	63.8	62.5	180.5	179.6	180.0	0.6
PPG-DH	6.9	6.4	4.5	4.2	0.7	0.7	65.2	65.6	181.4	181.7	181.6	0.2

10% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.2	5.8	2.2	2.0	0.4	0.3	35.5	34.5	155.0	153.8	154.4	0.9
Bovine serum albumin (66 kDa)	6.2	5.8	4.1	3.7	0.7	0.6	66.1	63.8	182.0	180.5	181.3	1.1
Ovalbumin (45 kDa)	6.2	5.8	4.3	4.1	0.7	0.7	69.4	70.7	184.1	184.9	184.5	0.6
Trypsin inhibitor type (21 kDa)	6.2	5.8	6.2	5	1.0	0.9	100.0	86.2	200.0	193.6	196.8	4.6
PEG-DH	6.2	5.8	3.7	3.5	0.6	0.6	59.7	60.3	177.6	178.1	177.9	0.3
PPG-DH	6.2	5.7	4.1	3.8	0.7	0.7	66.1	66.7	182.0	182.4	182.2	0.2

11% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	6.2	6.1	1.9	1.9	0.3	0.3	30.6	31.2	148.6	149.3	149.0	0.5
Bovine serum albumin (66 kDa)	6.2	6.1	3.7	3.6	0.6	0.6	59.7	59.0	177.6	177.1	177.3	0.3
Ovalbumin (45 kDa)	6.2	6.1	4.0	4.0	0.6	0.7	64.5	65.6	181.0	181.7	181.3	0.5
Trypsin inhibitor type (21 kDa)	6.2	6.1	6.2	6.1	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	6.2	6.1	3.1	3.0	0.5	0.5	50.0	49.2	169.9	169.2	169.5	0.5
PPG-DH	6.2	6.1	3.6	3.5	0.6	0.6	58.1	57.4	176.4	175.9	176.1	0.4

12% acrylamide gel

Standard protein	Dye (cm)		Band (cm)		Rf		Rf x 100		100 log (Rf x 100)			
	1	2	1	2	1	2	1	2	1	2	Mean	SD
<i>holo</i> -Transferrin (80 kDa)	5.9	5.8	1.6	1.6	0.3	0.3	27.1	27.6	143.3	144.1	143.7	0.5
Bovine serum albumin (66 kDa)	5.9	5.8	3.1	3.0	0.5	0.5	52.5	51.7	172.1	171.4	171.7	0.5
Ovalbumin (45 kDa)	5.9	5.8	3.5	3.5	0.6	0.6	59.3	60.3	177.3	178.1	177.7	0.5
Trypsin inhibitor type (21 kDa)	5.9	5.8	5.9	5.8	1.0	1.0	100.0	100.0	200.0	200.0	200.0	0
PEG-DH	5.9	5.8	2.7	2.6	0.5	0.4	45.8	44.8	166.1	165.2	165.6	0.6
PPG-DH	5.9	5.8	3.1	3.0	0.5	0.5	52.5	51.7	172.1	171.4	171.7	0.5

BIOGRAPHY

Miss Thanida Vuthikulvanich was born on July 29, 1981 in Bangkok, Thailand. She finished High School at Triumudomsuksa Patthanakarn School, Bangkok and enrolled in the Faculty of Science, Chulalongkorn University in 1999. She graduated with the Bachelor Degree of Science in Biochemistry in 2003 and continued studying for Master Degree of Science in Biotechnology in that year. She finished Master Degree of Science in Biotechnology in May 2007. She has publication:

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