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APPENDICES

A. Activities of trypsin and dispase II

The activity assay of enzyme was assayed at 30°C and pH 7.4. 0.9 ml of 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of 1% (w/v) trypsin solution or 0.48% (w/v) dispase II were mixed in a test tube and shaken by a vortex mixer. 1 ml of 0.2% (w/v) azo-casein solution was added to the mixing solution and further shaken. The solution was incubated in a water bath at 30°C for 20 minutes. After that, 2 ml of 10% (w/v) trichloroacetic acid solution was added to the solution for precipitation of non-hydrolyzed substrate, and then the solution was put in a ice bath at about 4°C to stop the reaction. Finally, the solution was centrifuged at 3,000 rpm (1,660g) for 20 minutes to separate the solid and liquid phases. The absorbance of the sample at the wavelength of 440 nm was measured using a UV-VIS spectrophotometer. Blank test was prepared by adding the TCA solution to the substrate before azo-casein solution was added.

Table A-1 The absorbance of enzyme solutions at the wavelength of 440 nm

Replication no.	Absorbance	
	Trypsin (0.25% w/v)	Dispase II (0.112% w/v)
1	0.028	0.056
2	0.03	0.055
3	0.027	0.053
Average	0.028	0.054
SD	0.0012	0.009

The activity of enzyme is calculated from the change of absorbance at the wavelength of 440 nm (the absorbance increases by 0.1, the unit of activity increases 1). For trypsin, 0.1 ml of enzyme solution has an absorbance 0.028. The activity of 0.1 ml of enzyme solution is 0.28 unit. Therefore, the activity of 1 ml of enzyme solution is 2.8 unit/ml. When comparing with the incubation time (20 minutes), the activity in one minute is 0.14 unit/ml. The calculation is showed as follows:

Activity of 0.25% (w/v) trypsin solution at 30°C, pH 7.4

Activity of 0.1 ml of enzyme solution	=	(0.028)*10 = 0.28	unit
Activity of 1 ml of enzyme solution	=	$\frac{1*0.28}{0.1} = 2.8$	unit/ml
Activity in one minute	=	$\frac{2.8}{20} = 0.14$	unit/ml

Activity of 0.112% (w/v) dispase II solution at 30°C, pH 7.4

Activity of 0.1 ml of enzyme solution	=	(0.054)*10 = 0.54	unit
Activity of 1 ml of enzyme solution	=	$\frac{1*0.54}{0.1} = 5.4$	unit/ml
Activity in one minute	=	$\frac{5.4}{20} = 0.27$	unit/ml

The total activity of enzyme is calculated from the absorbance of the wavelength of 440 nm of 0.25% (w/v) trypsin and 0.112% (w/v) dispase II. The activity, activity per gram, and total activity of trypsin and dispase II are presented in Table A-2, A-3, and A-4, respectively

Table A-2 The activity of enzyme solutions

Type of enzyme solution	Trypsin (0.25%w/v)	Dispase II (0.112%w/v)
Activity (unit/ml)	0.14	0.27

Table A-3 The activity per gram of enzymes

Type of enzyme	Trypsin (1g)	Dispase II (1g)
Activity (unit/g)	56	241

Table A-4 The total activity of enzyme

Type of enzyme	Trypsin (2g)	Dispase II (0.48g)
Total activity (unit)	112	115

B. Standard curve of DNA assay

Standard curve of DNA assay was prepared using L929 mouse fibroblasts. A series of L929 mouse fibroblasts at the concentration of 500,000, 250,000, 125,000, 62,500, 31,250 cells/ml were used to lyses cell. The mixture was incubated at 55°C for 6 hr with occasional mixing. After incubation, centrifugation was performed at 2,236g (10,000 rpm) for 10 min. Fluorescence intensity was determined with a fluorescence spectrophotometer (VICTOR^3 Perkin-elmer USA) at the excitation and emission wavelengths of 355 and 460 nm, respectively.

Table B-1 Fluorescence count of L929 mouse fibroblasts at the excitation and emission wavelengths of 355 and 460 nm, respectively.

Replication no.	Fluorescence count					
	Blank	31,250 cells/ml	62,500 cells/ml	125,000 cells/ml	250,000 cells/ml	500,000 cells/ml
1	34,043	36,900	47,779	65,694	92,033	134,194
2	36,456	37,787	50,784	69,232	89,783	133,743
3	37,600	37,806	46,856	66,379	92,421	140,543
Average	36,033	37,508	48,473	67,102	91,412	136,150
SD	1,816	500	2,054	1,876	1,424	3,785

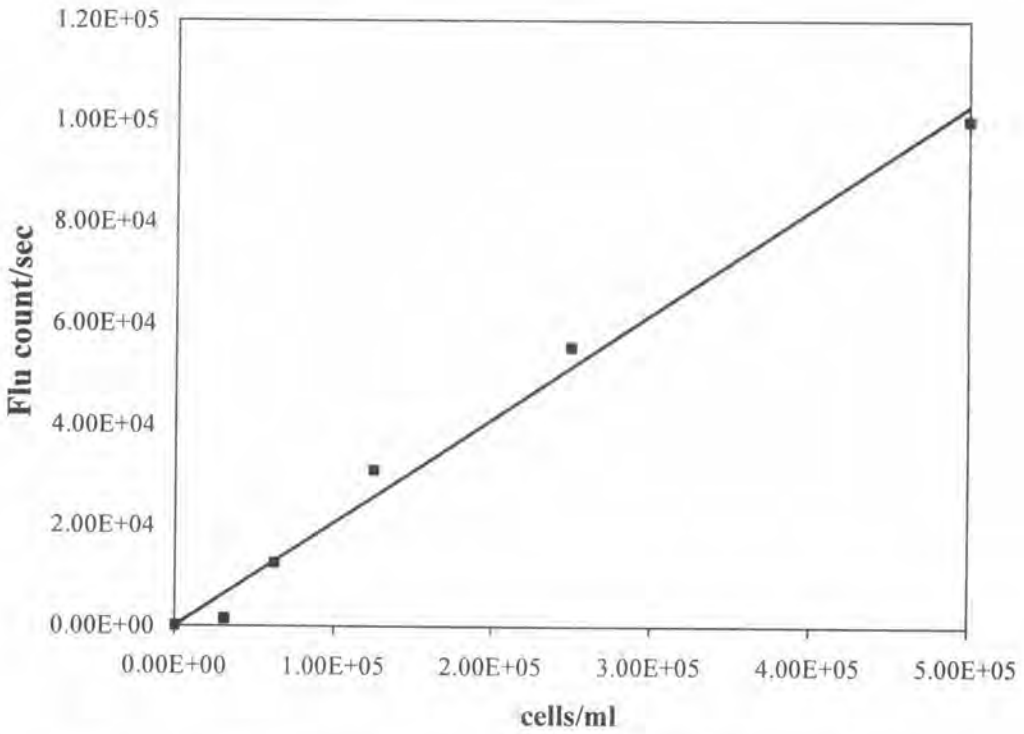

Figure B-1 Standard curve of DNA assay using L929 mouse fibroblasts.

VITAE

Mr. Isarawut Prasertsung was born in Roi-Et, Thailand on January 31, 1980. He finished high school in 1998 from Roi-Et Wittayalai School. In 2002, he graduated from Faculty of Engineering, Rajamangala Institute of Technology with a Bachelor of Engineering in Plastic Engineering. After the graduation, he pursued his graduate study to a Master of Engineering (Chemical Engineering), Faculty of Engineering, Chulalongkorn University.