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APPENDIX I

1. Coating buffer (Carbonate-bicarbonate buffer pH 9.6)

Na_2CO_3	1.59	g.
$NaHCO_3$	2.93	g.
Distilled water	1000	ml.

The pH was adjusted to 9.6 and stored at 4°C for not more than 2 weeks.

2. Washing buffer (PBS-Tween PH 7.4)

NaCl	8.0	g.
KH_2PO_4	0.2	g.
$Na_2HPO_4 .12H_2O$	2.9	g.
KCl	0.2	g.
Tween 20	0.5	ml.
distilled water	1000	ml.

The pH was adjusted to 7.4 and stored at 4°C

3. Sample and conjugate diluent (PBS-Tween albumin)

Bovine serum albumin (BSA)	1	g.
PBS-Tween	100	ml.

Stored at 4°C.

4. Substrate buffer (Citric acid phosphate buffer pH 5.0)

Citric acid H2O	7.30	g.
Na2HPO4	4.97	g.
Distilled water	1000	ml.

The pH was adjusted to 5.0 and stored at 4°C

5. Substrate solution

O-phenylenediamine dihydrochloride	8	g.
Substrate buffer	12	ml.
30% H_2O_2	5	ul.

It must be freshly prepared before used and should be protected from strong light.

6. Stopping solotion (1 N H_2SO_4)

H_2SO_4 Conc.	28	ml.
Distilled water to	1000	ml.

Stored at room temperature

APPENDIX II

Table 1 Checkerboard titration to determine the optimal dilutions of serum and anti-human IgG peroxidase conjugate.

Anti-human IgG peroxidase conjugate at a dilution of	Absorbance value at serum dilution of			
	1:200	1:400	1:800	1:1600
1:5000	3.355	2.264	2.075	1.405
1:10000	3.085	2.168	1.953	1.349
1:15000	3.101	1.957	1.447	1.207
1:20000	2.728	1.549	1.161	0.947
1:5000	0.379	0.210	0.148	0.116
1:10000	0.258	0.136	0.098	0.076
1:15000	0.173	0.089	0.067	0.046
1:20000	0.228	0.070	0.049	0.035

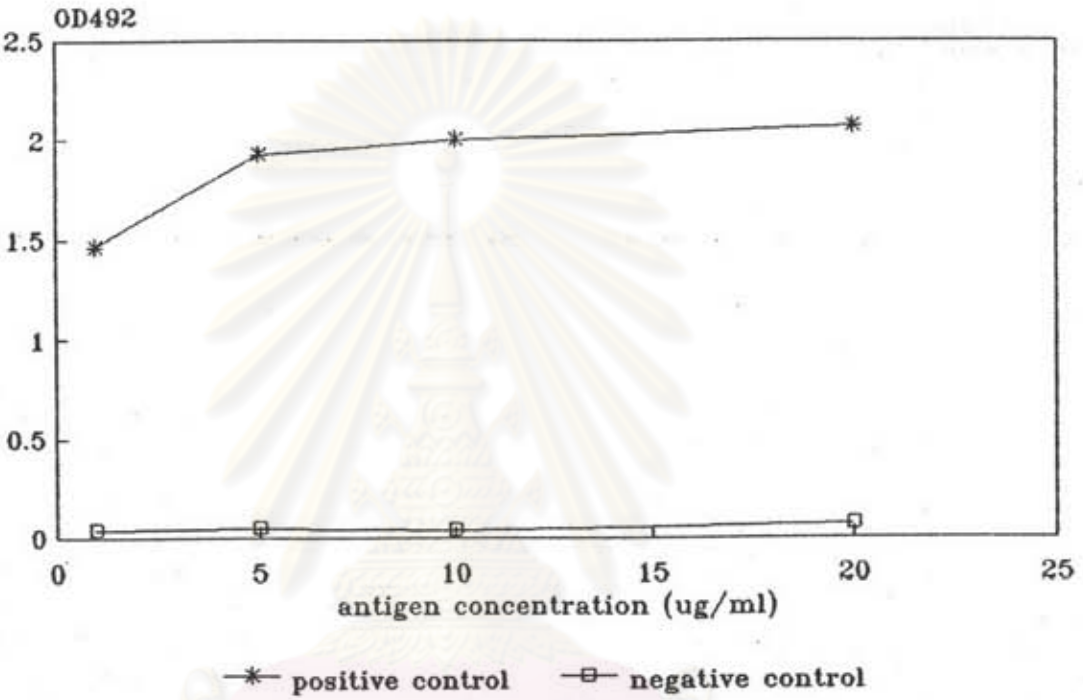

Figure 1 Determination of the optimal concentration of antigen

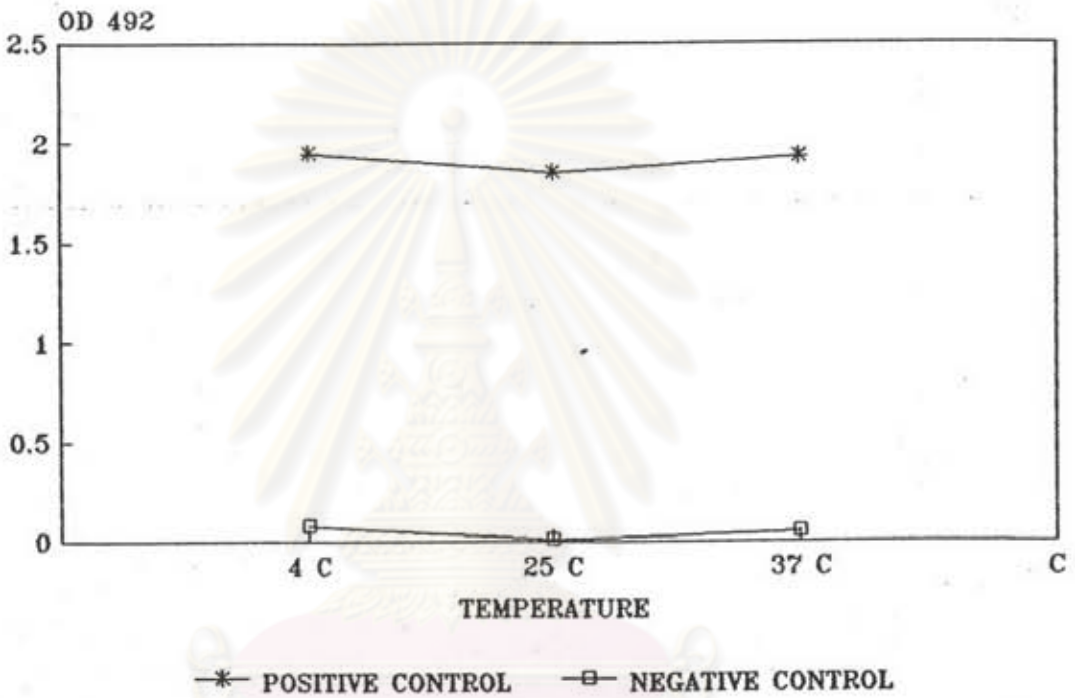

Figure 2 Determination of the optimal temperature for antigen incubation.

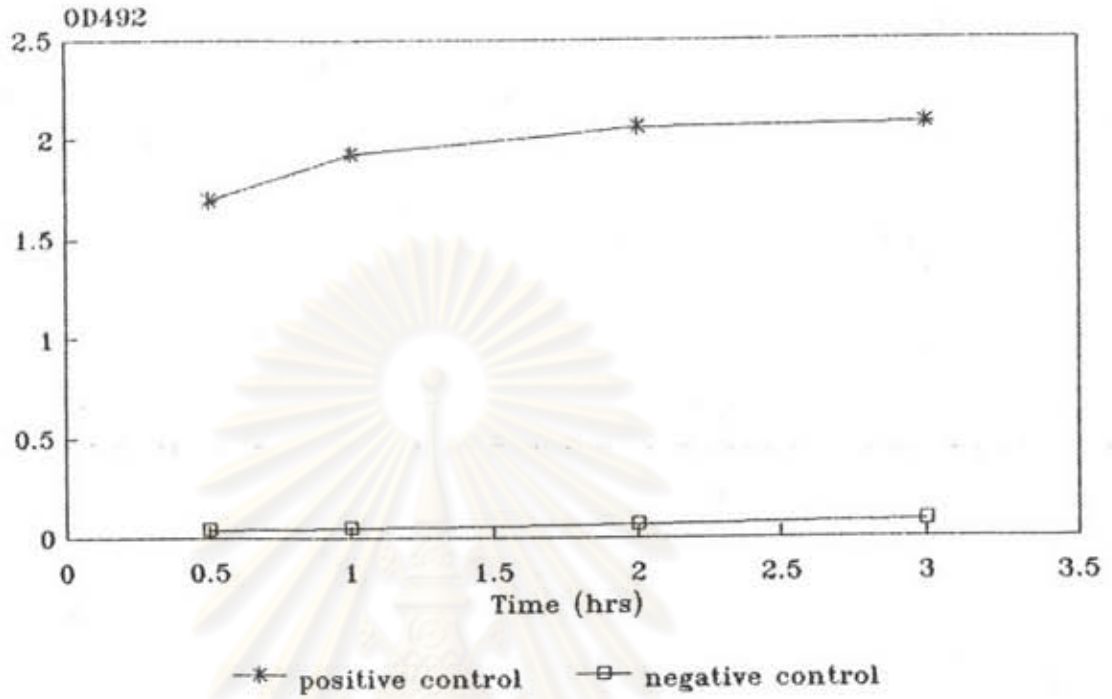

Figure 3 Determination of the optimal time for antigen incubation.

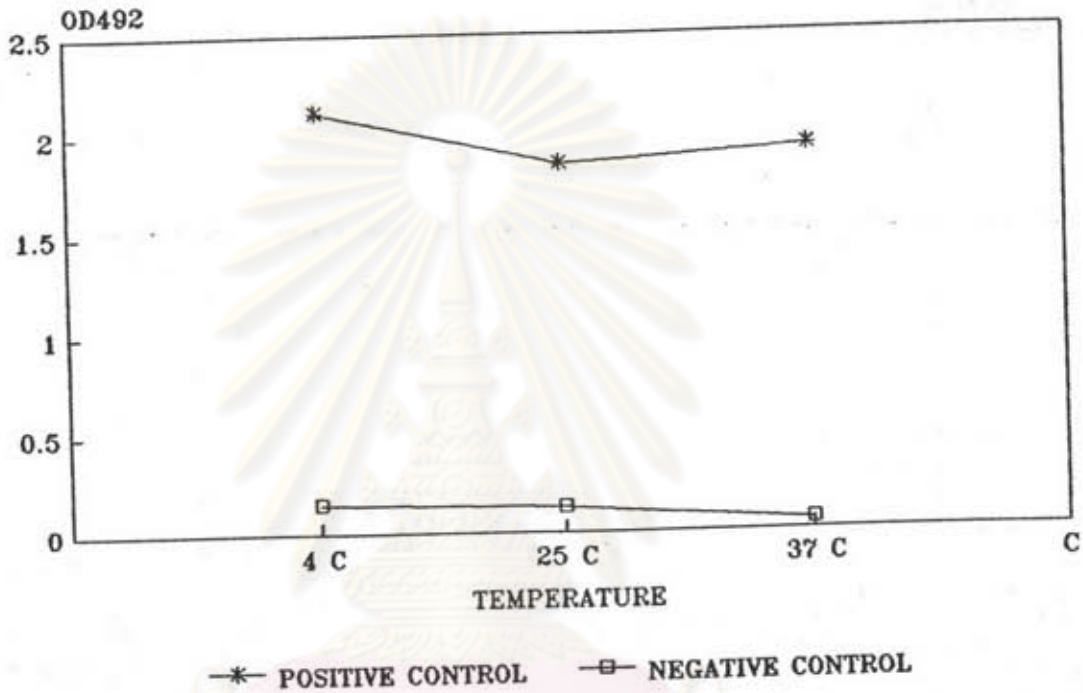

Figure 4 Determination of the optimal temperature for serum incubation.

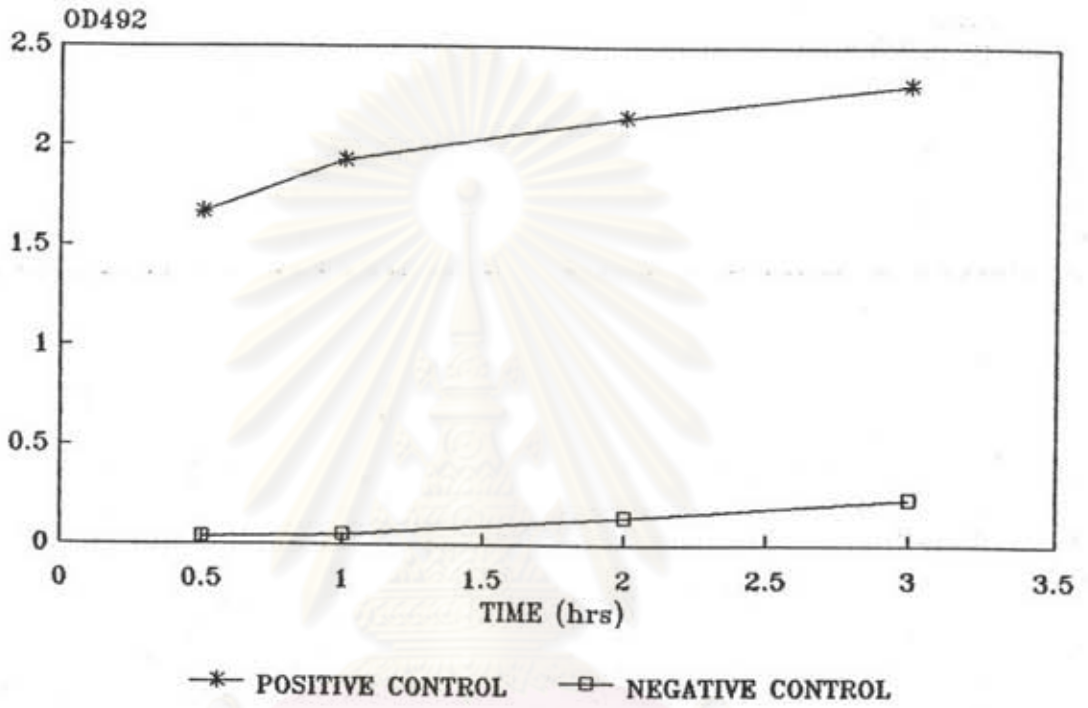

Figure 5 Determination of the optimal time for serum incubation.

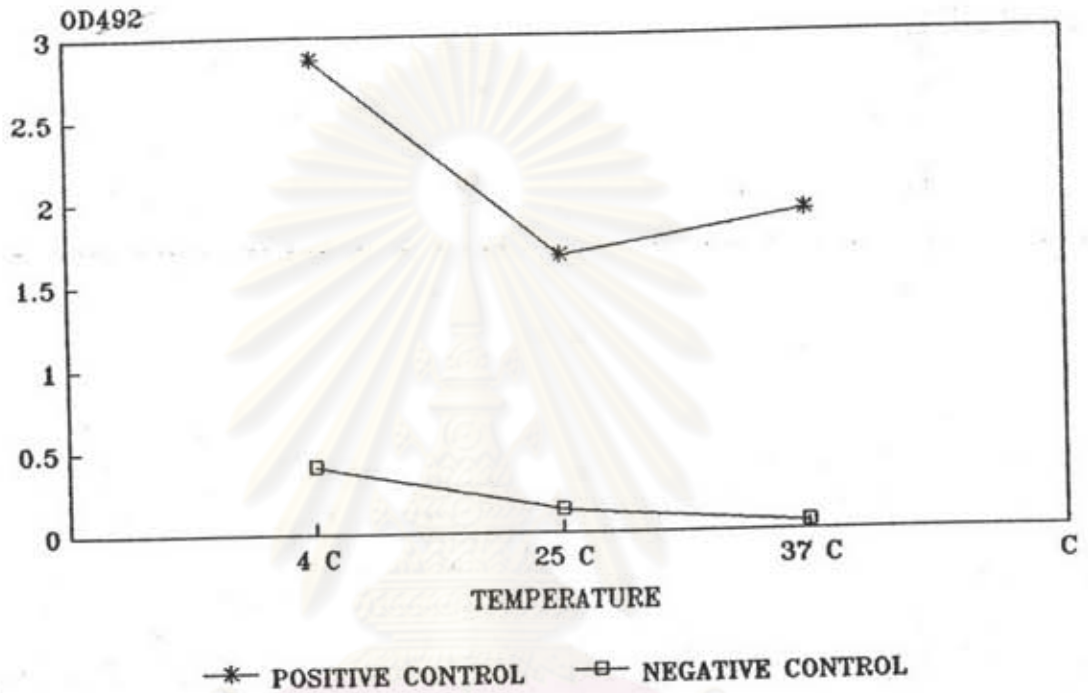

Figure 6 Determination of the optimal temerature for conjugate incubation.

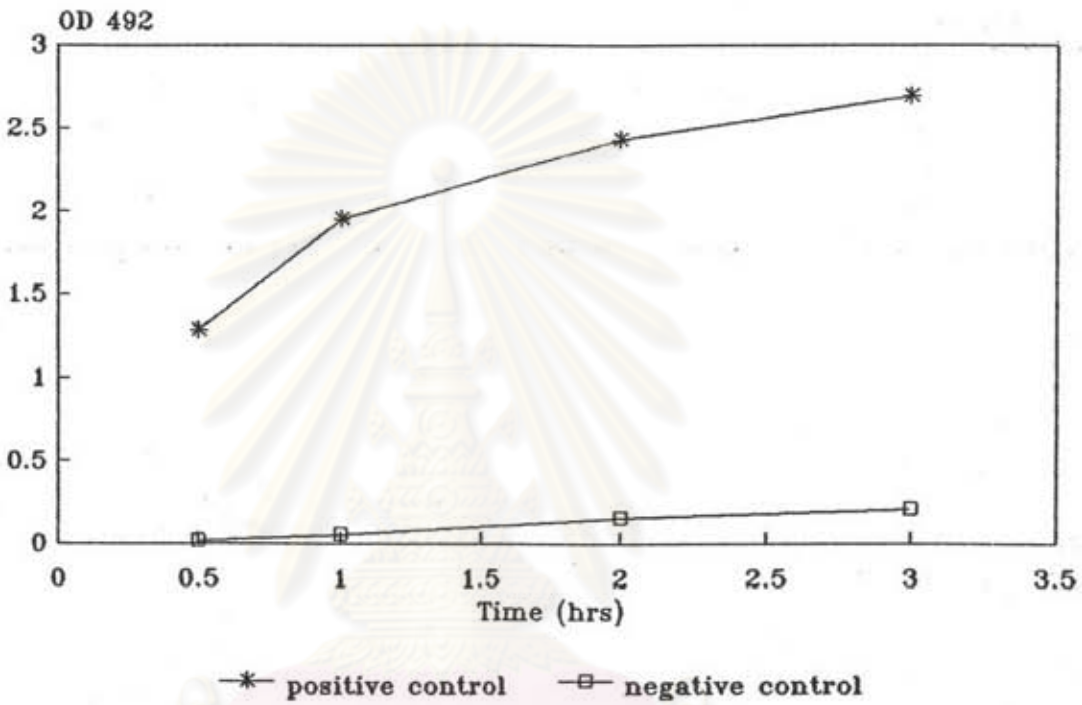

Figure 7 Determination of the optimal time for conjugate incubation.

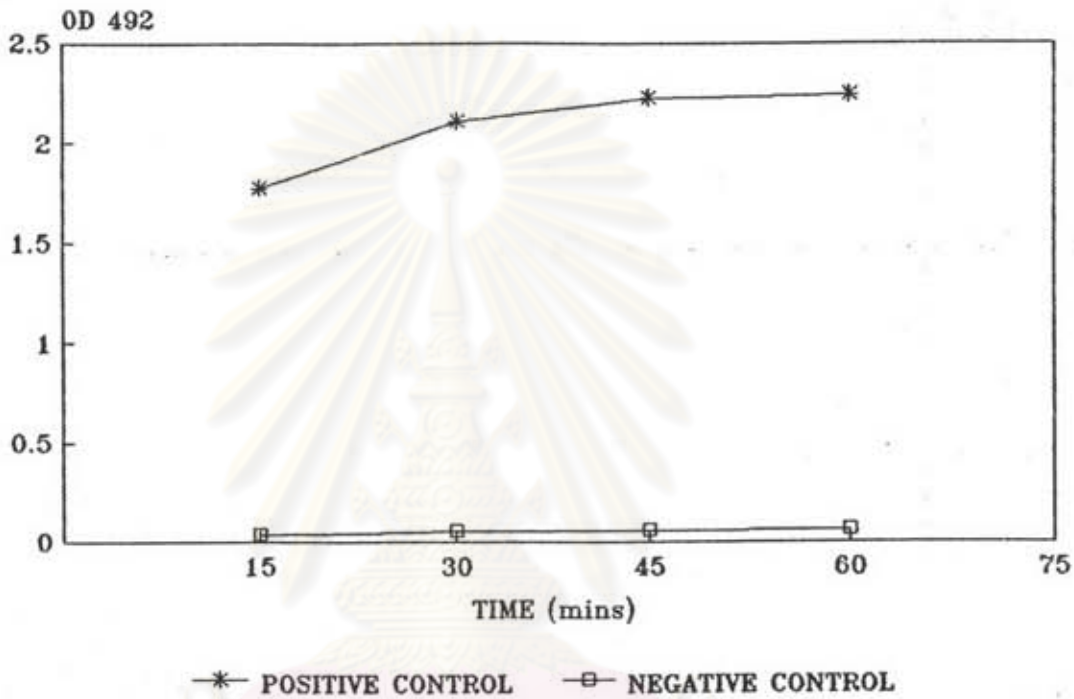

Figure 8 Determination for the optimal time for substrate incubation.

Table 2 Checkerboard titration to determine the optimal dilutions of serum and anti-human IgE peroxidase conjugate.

Anti-human IgE peroxidase conjugate at a dilution of	Absorbance value at serum dilution of			
	1:25	1:50	1:100	1:200
1:250	0.571	0.541	0.350	0.241
1:500	0.552	0.538	0.323	0.227
1:1000	0.459	0.392	0.260	0.191
1:2000	0.412	0.321	0.316	0.190
1:250	0.173	0.087	0.068	0.051
1:500	0.167	0.073	0.056	0.050
1:1000	0.163	0.066	0.049	0.048
1:2000	0.158	0.063	0.041	0.035

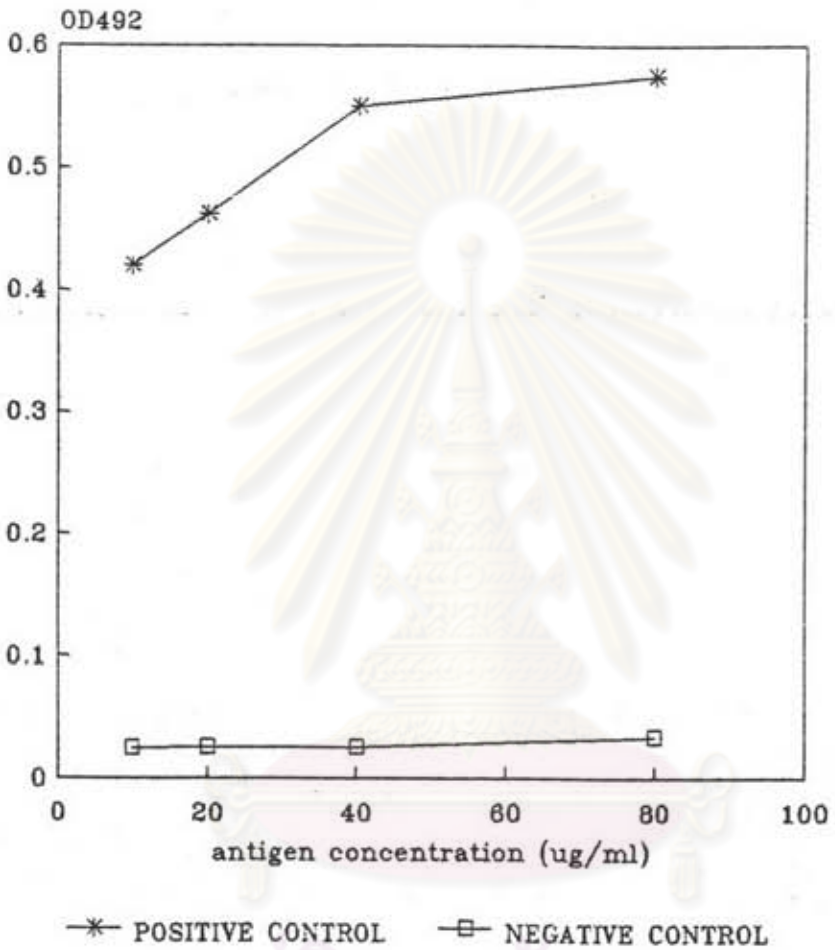

Figure 9 Determination of the optimal concentration of antigen.

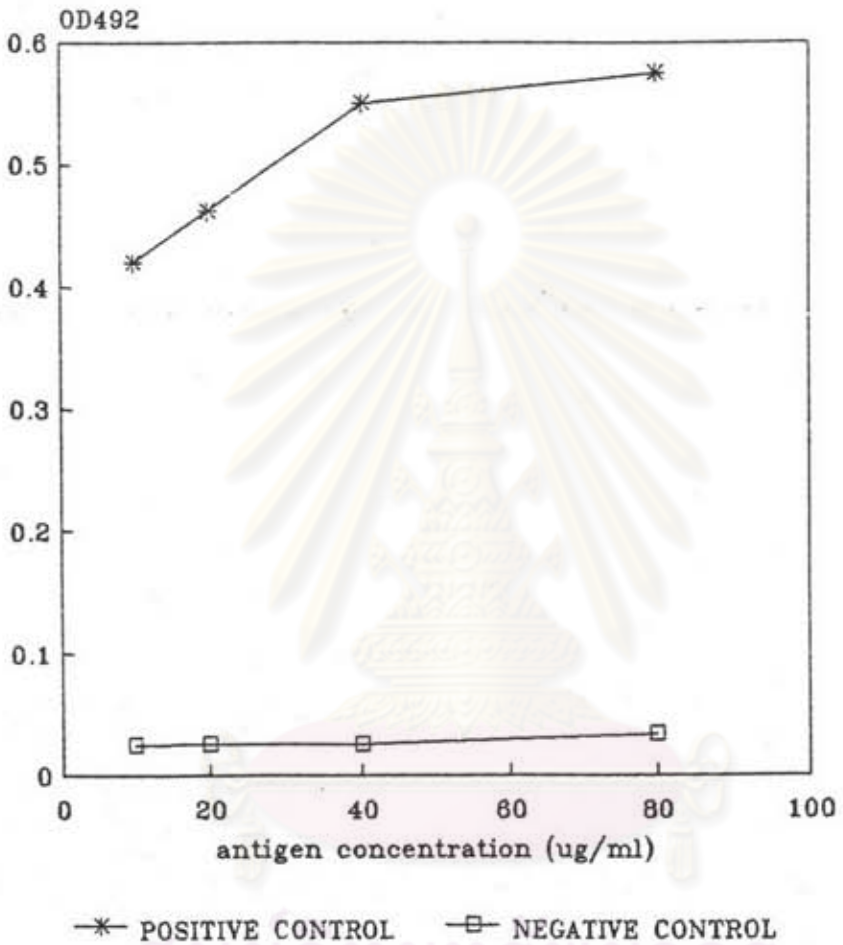

Figure 9 Determination of the optimal concentration of antigen.

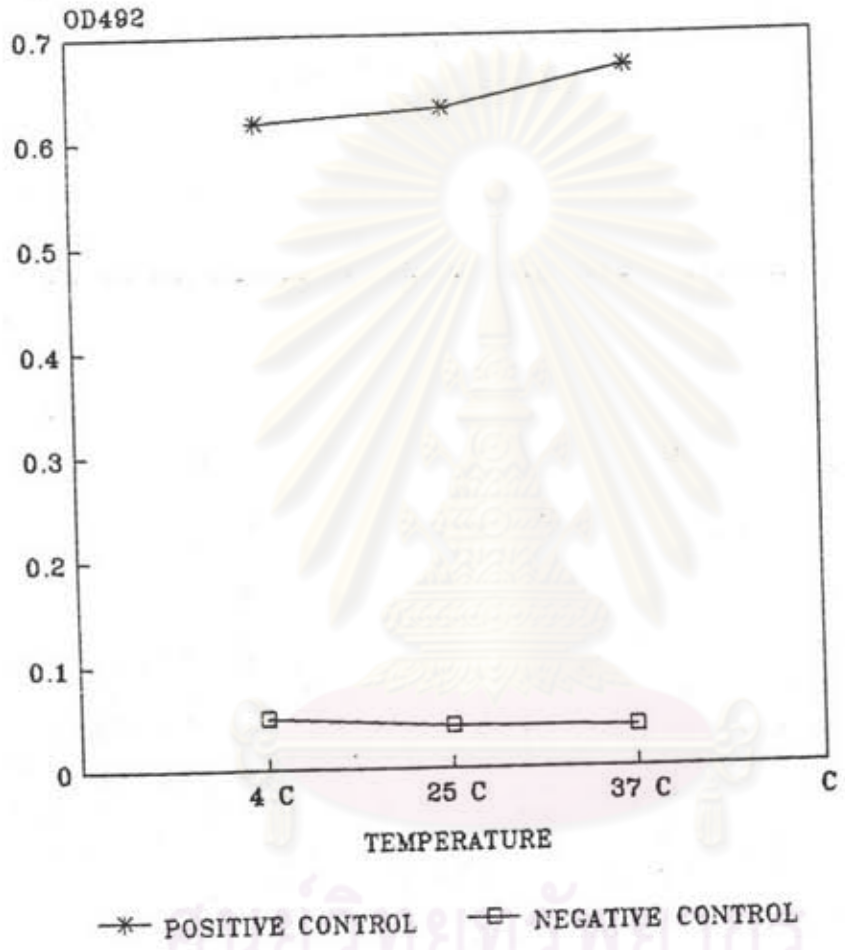

Figure 10 Determination of the optimal temperature for antigen incubation.

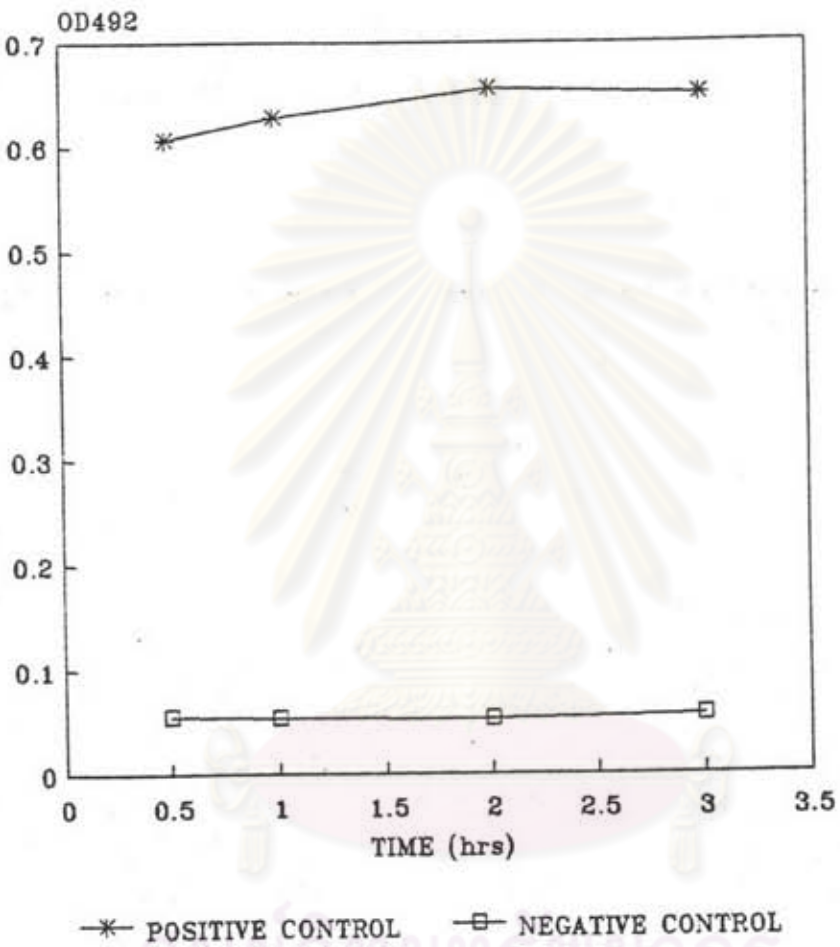

Figure 11 Determination of the optimal time for antigen incubation.

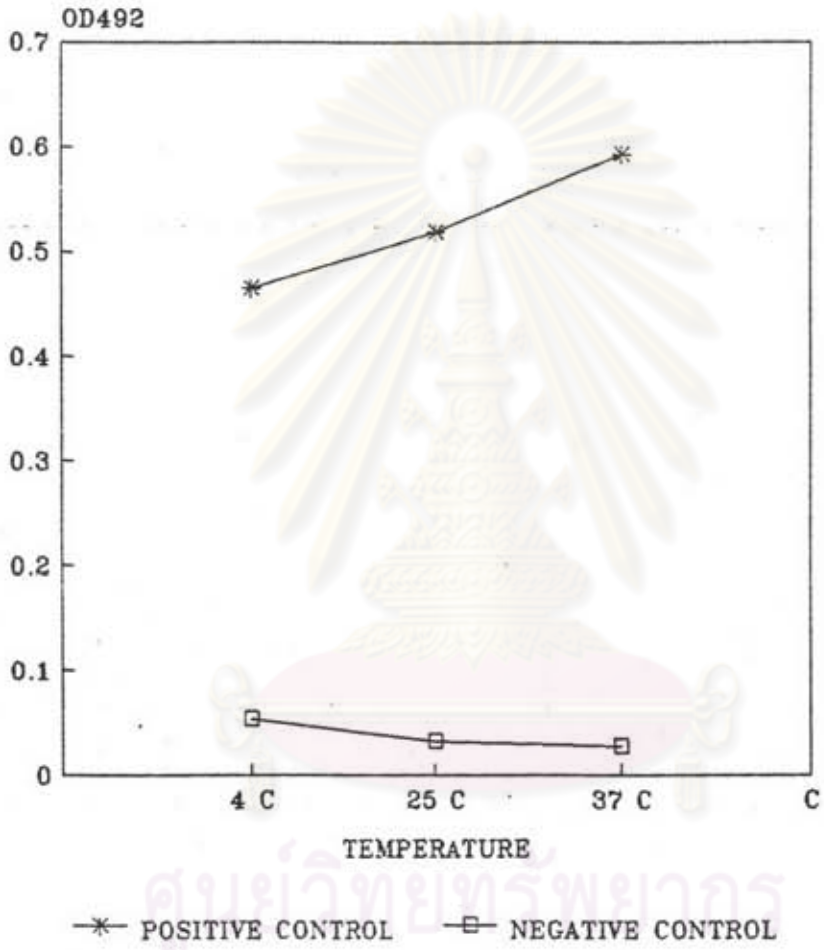

Figure 12 Determination of the optimal temperature for serum incubation.

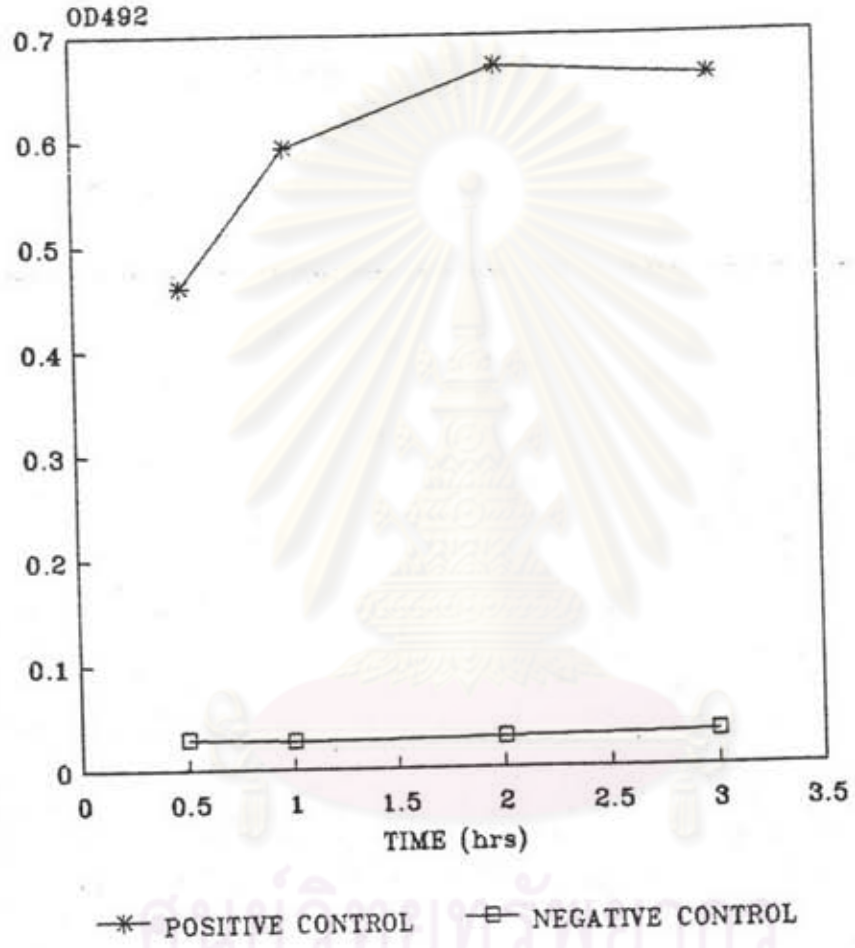

Figure 13 Determination of the optimal time for serum incubation.

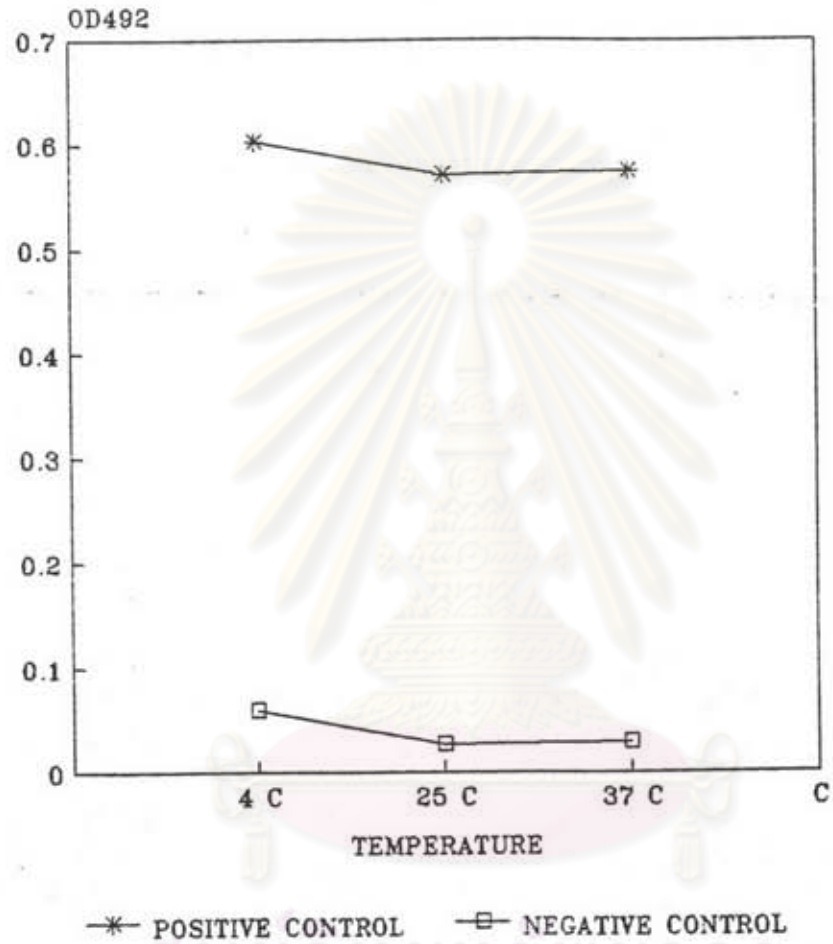

Figure 14 Determination of the optimal temperature for conjugate incubation.

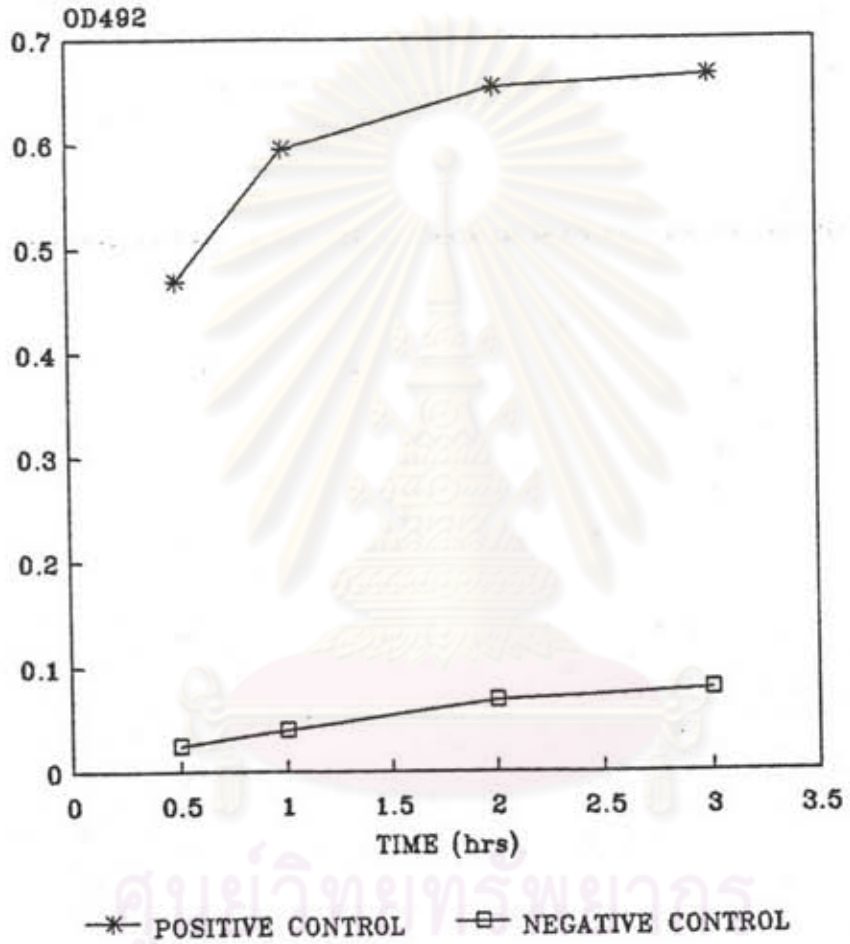

Figure 15 Determination of the optimal time for conjugate incubation.

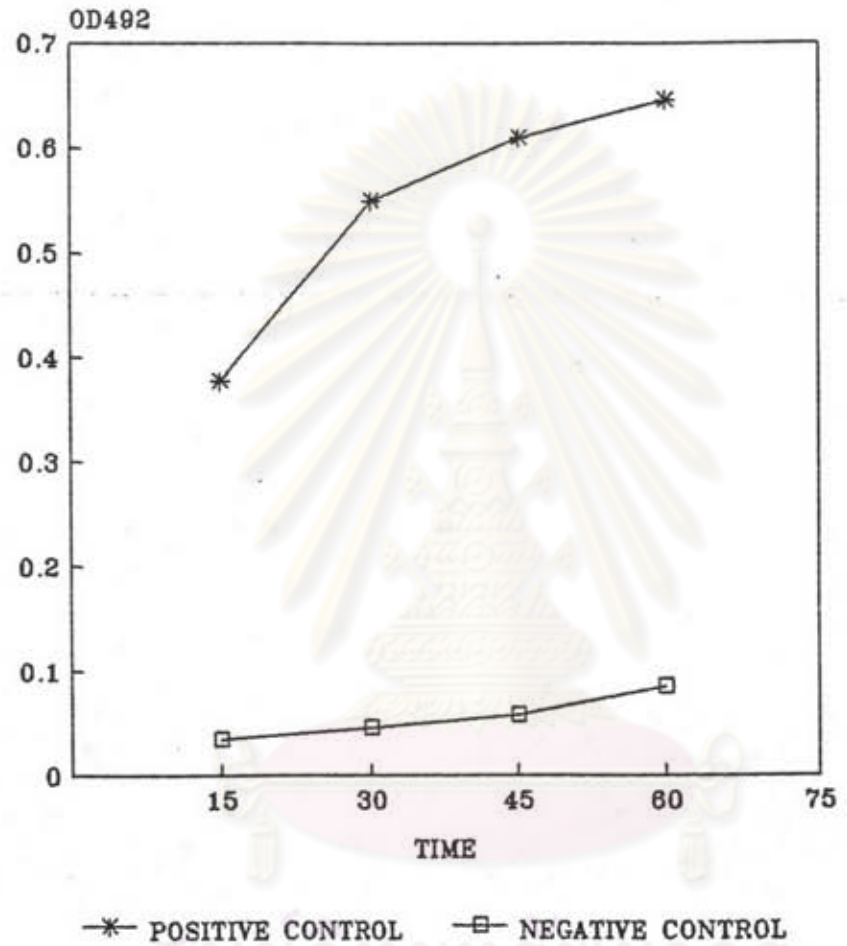

Figure 16 Determination of the optimal time for substrate incubation.

Table 3 Checkerboard titration to determine the optimal dilutions of serum and anti-human IgM peroxidase conjugate.

Anti-human IgM peroxidase conjugate at a dilution of	Absorbance value at serum dilution of			
	1:5	1:25	1:50	1:100
1:250	0.615	0.528	0.478	0.363
1:500	0.565	0.508	0.456	0.347
1:1000	0.496	0.471	0.411	0.321
1:2000	0.473	0.456	0.424	0.296
1:250	0.271	0.156	0.149	0.138
1:500	0.255	0.149	0.139	0.131
1:1000	0.215	0.141	0.127	0.121
1:2000	0.195	0.137	0.115	0.120

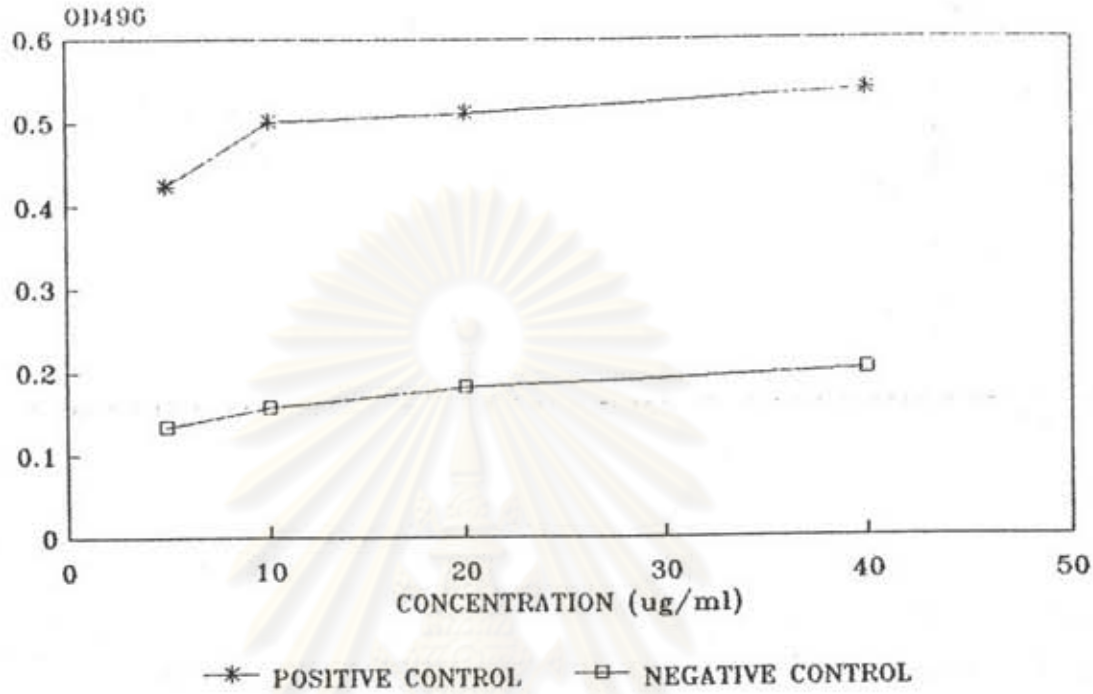

Figure 17 Determination of the optimal concentration of anti IgM antibody.

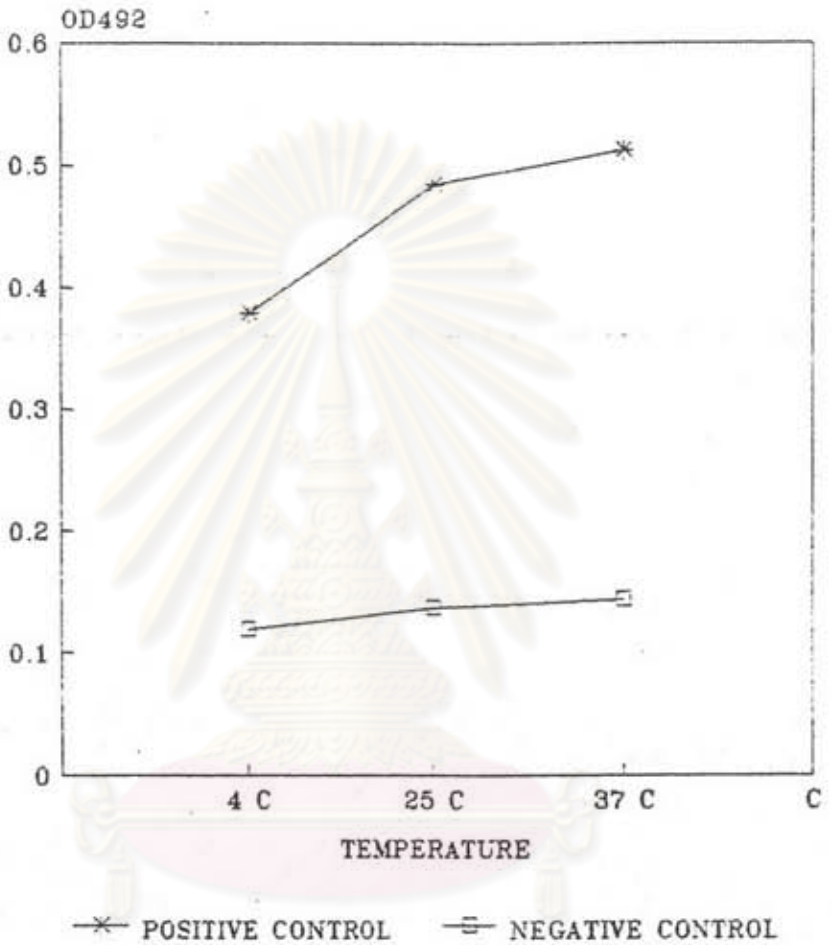

Figure 18 Determination of the optimal temperature for coating plate.

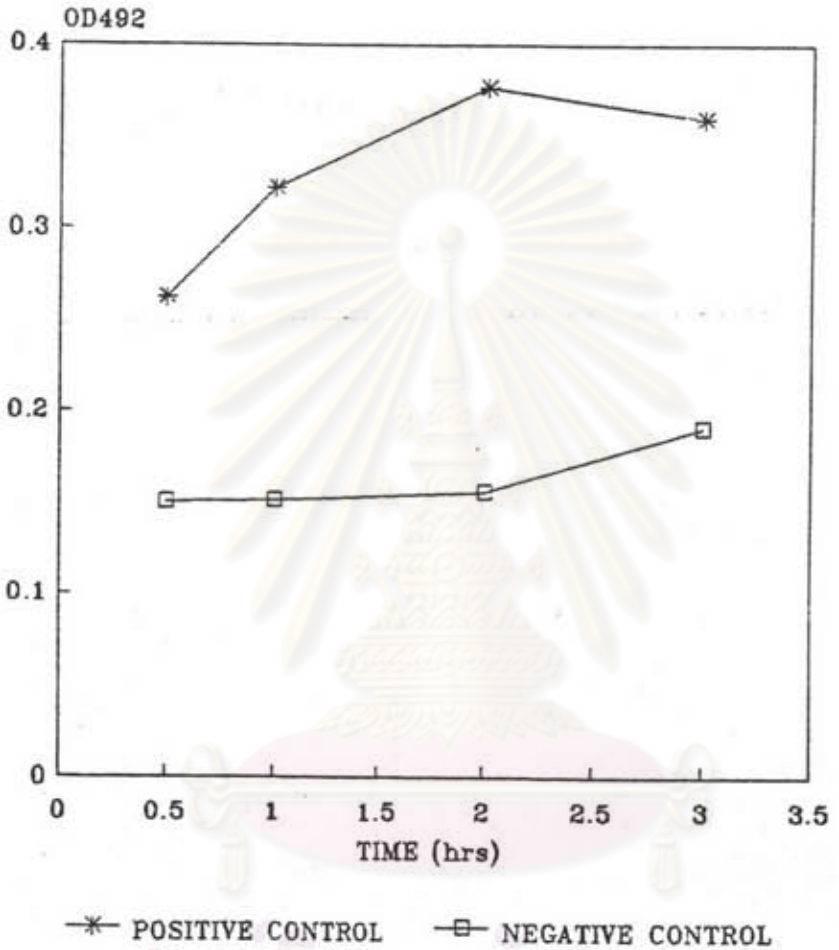

Figure 19 Determination of the optimal time for coating plate.

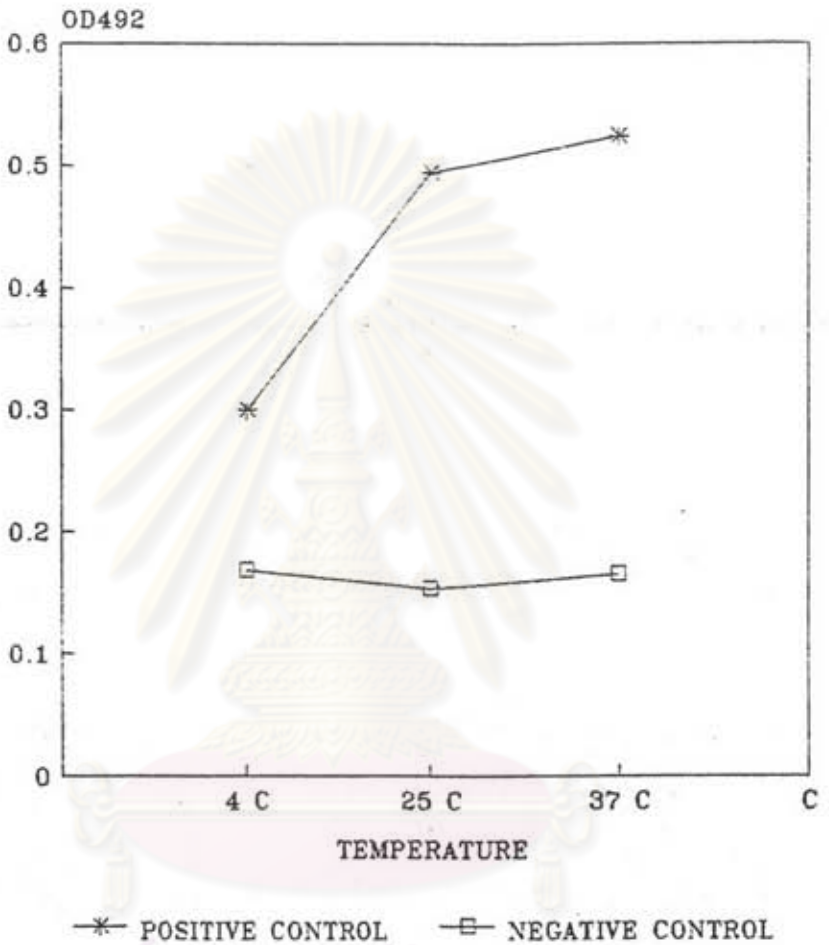

Figure 20 Determination of the optimal temperature for serum incubation.

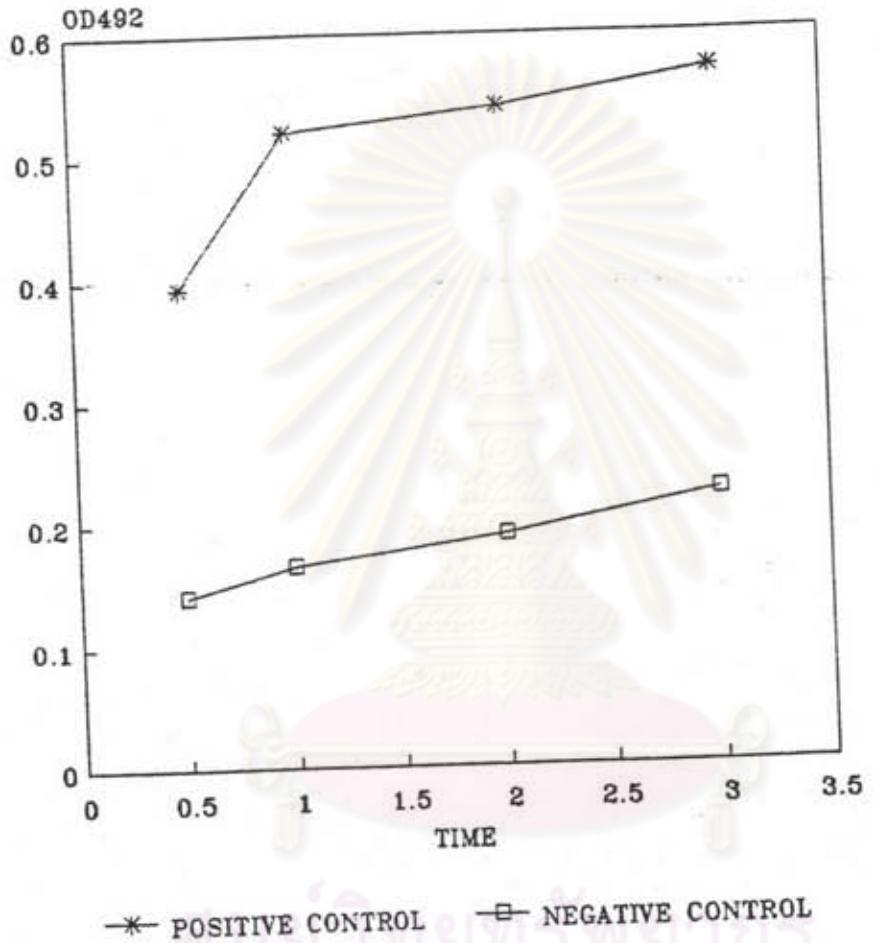

Figure 21 Determination of the optimal time for serum incubation.

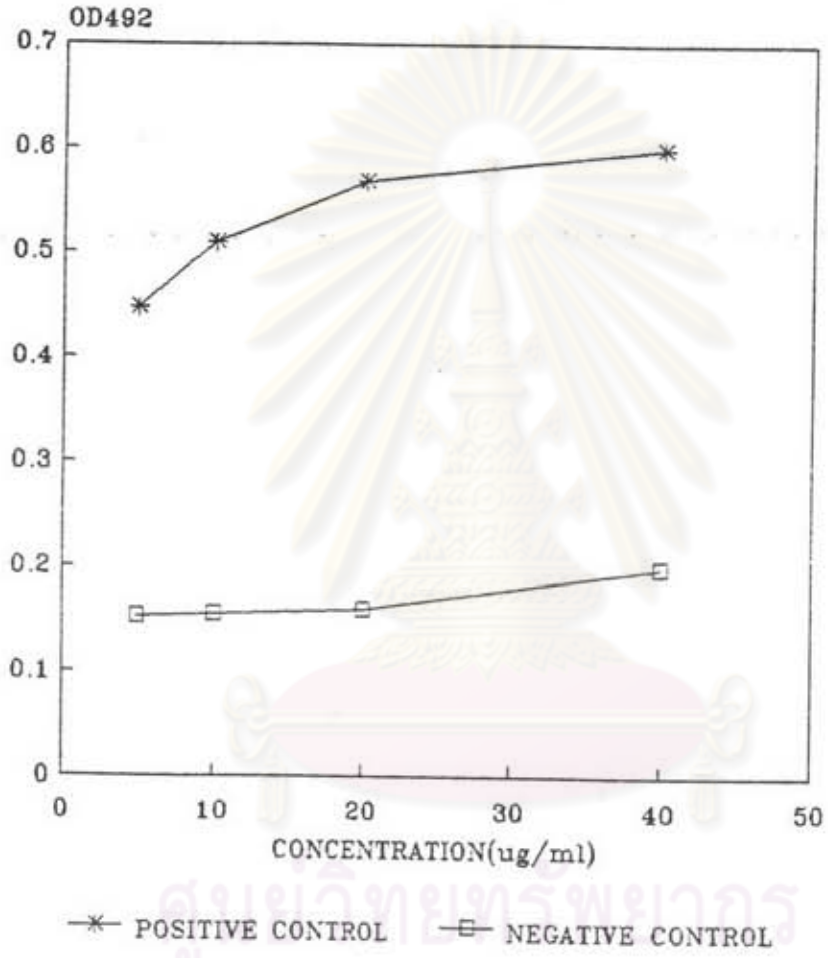

Figure 22 Determination of the optimal antigen concentration.

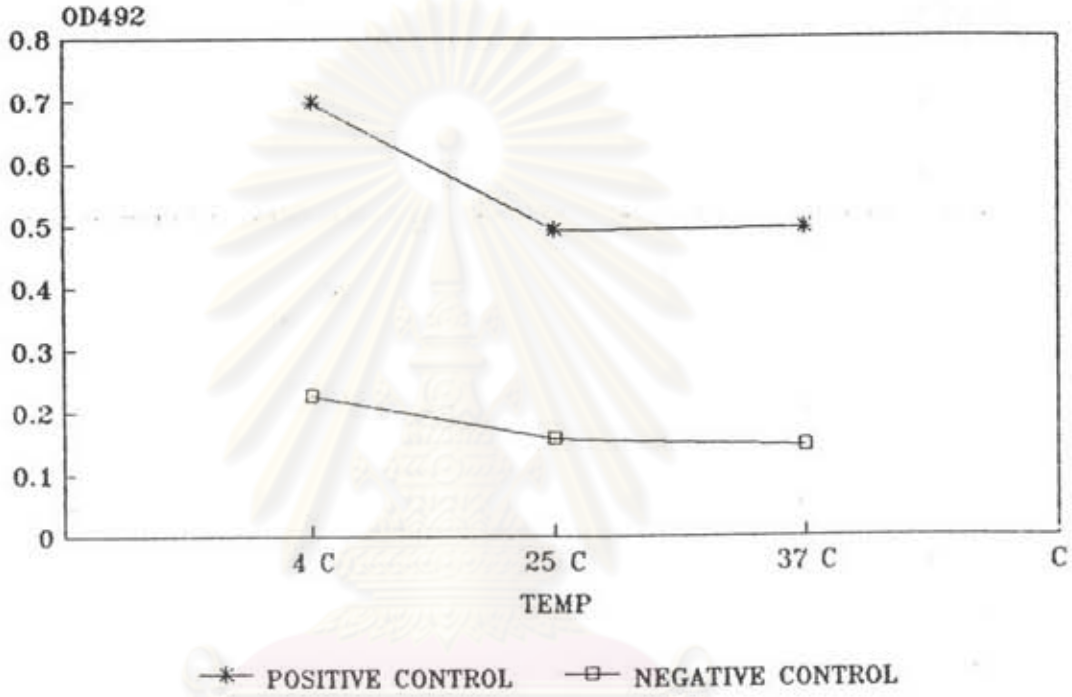

Figure 23 Determination of optimal temperature for antigen incubation.

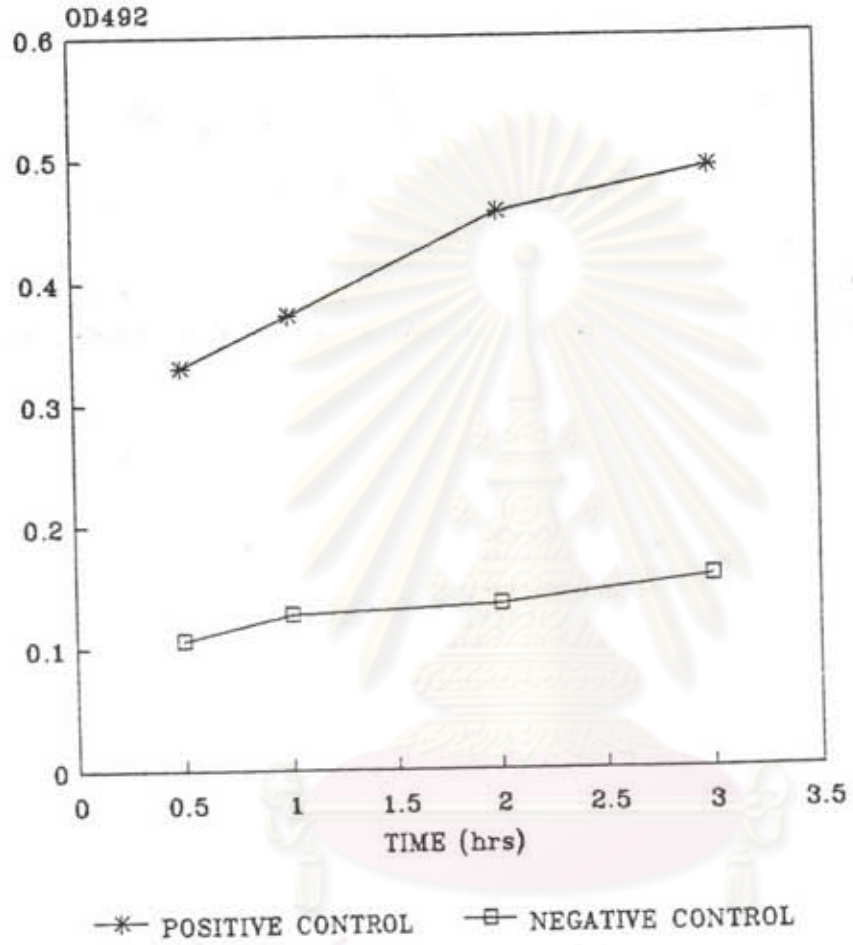

Figure 24 Determination of the optimal time for antigen incubation.

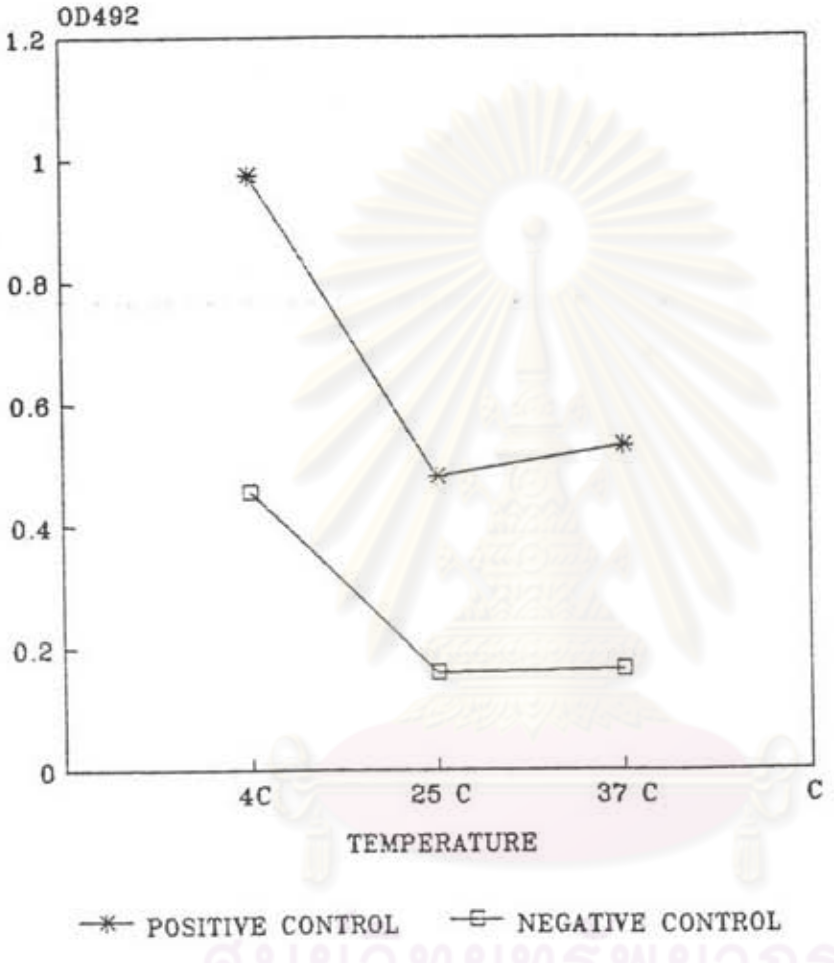

Figure 25 Determination of the optimal temperature for conjugate incubation.

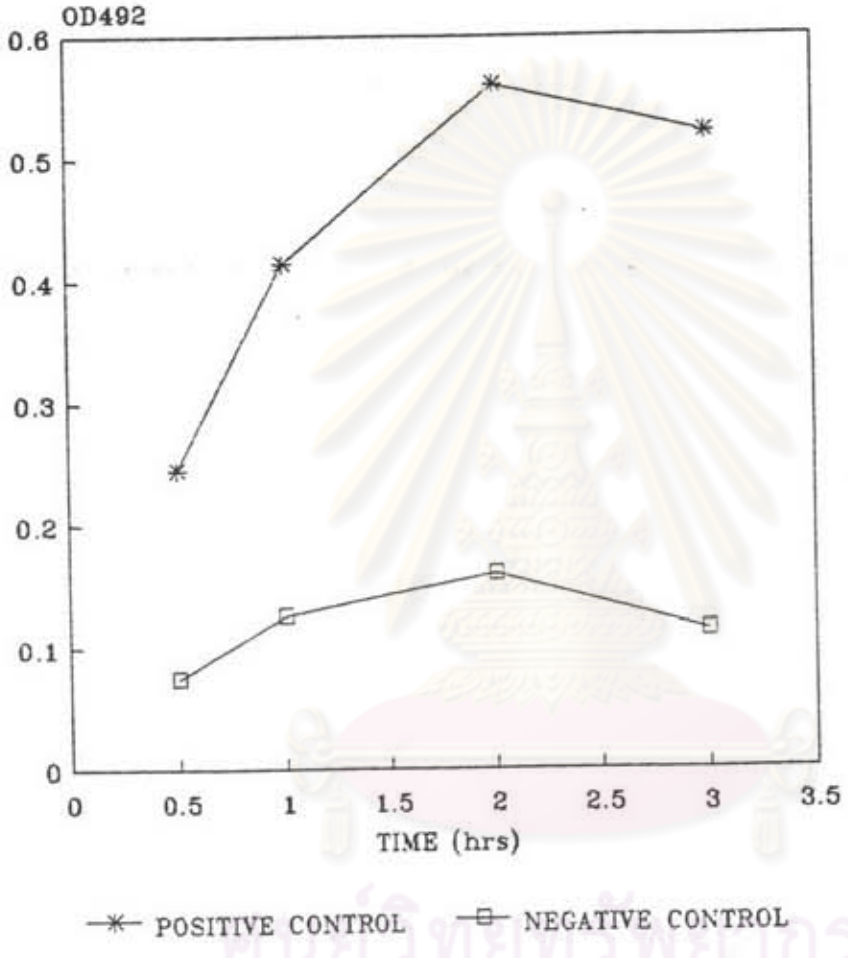

Figure 26 Determination of the optimal time for conjugate incubation.

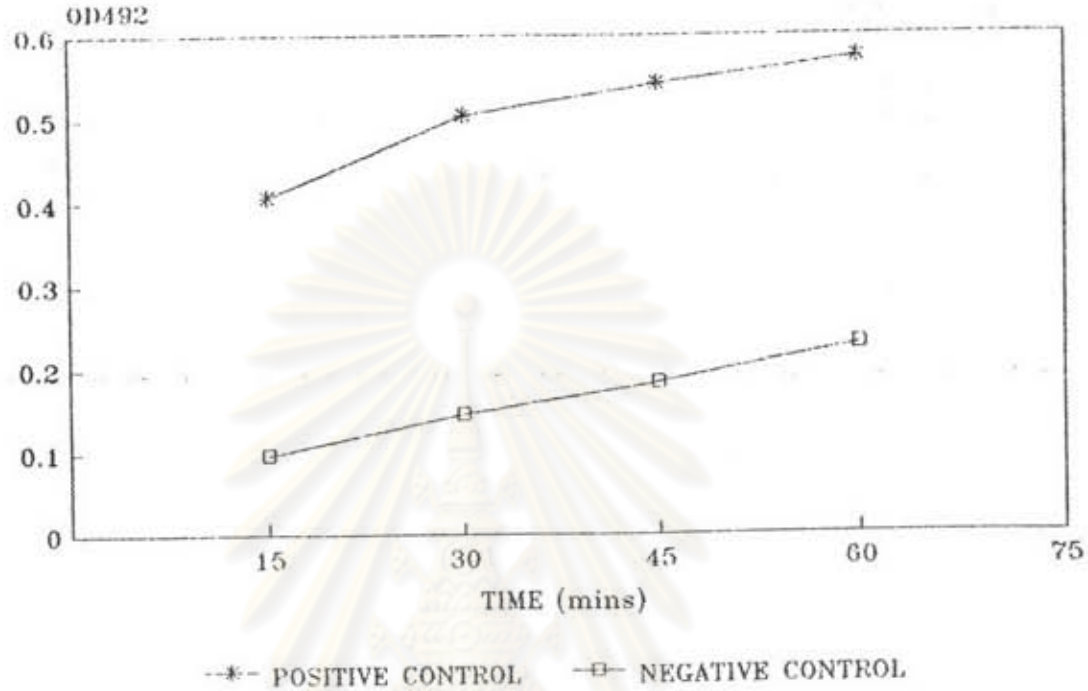

Figure 27 Determination of the optimal time for substrate incubation.

BIOGRAPHY

Miss Tanittha Chatsuwan was born on November 2, 1964 in Bangkok, Thailand. She graduated with a Bachelor degree of Science (Medical Technology) from the Faculth of Medicine at Chulalognkorn University in 1987.