

A vertical strip of a microplate with a pink triangle pointing to the right.

Spectrophotometry (Lowry assay)



Introduction

➤ Lowry assay

- ❖ 단백질 정량 분석 방법

- ❖ $5\mu\text{g}/\text{ml}$ 정도의 적은 양으로도 정량이 가능.

- ❖ 단백질 정제에 있어 단백질 농도 측정에 널리 쓰임.

- ❖ 분석가능범위 : $5\mu\text{g}/\text{ml} \sim 100\mu\text{g}/\text{ml}$

- ❖ 소요시간 : 약 40분

- ❖ 실험을 통해 미지의 시료 안에 있는 단백질의 절대량을 구할 수 있다.



Equipments

- ❖ Spectrophotometer
- ❖ Pipets
- ❖ Voltex mixer
- ❖ Test tube
- ❖ Test tube rack
- ❖ Cuvettes*

Reagents

Table 1. Reagents for experiment.

Solution	용 질	용 매	Total vol.
A	0.5g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 1g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7(2\text{H}_2\text{O})$	D.W	100ml
B	20g Na_2CO_3 4g NaOH	D.W	1liter
C	1ml Solution A 50ml Solution B	.	51ml
D	10ml Folin – Ciocalteu phenol reagent 10ml Distilled water	.	20ml
주 의 :	Solution A,B 는 상온저장, Solution C,D 는 측정 시 제조		

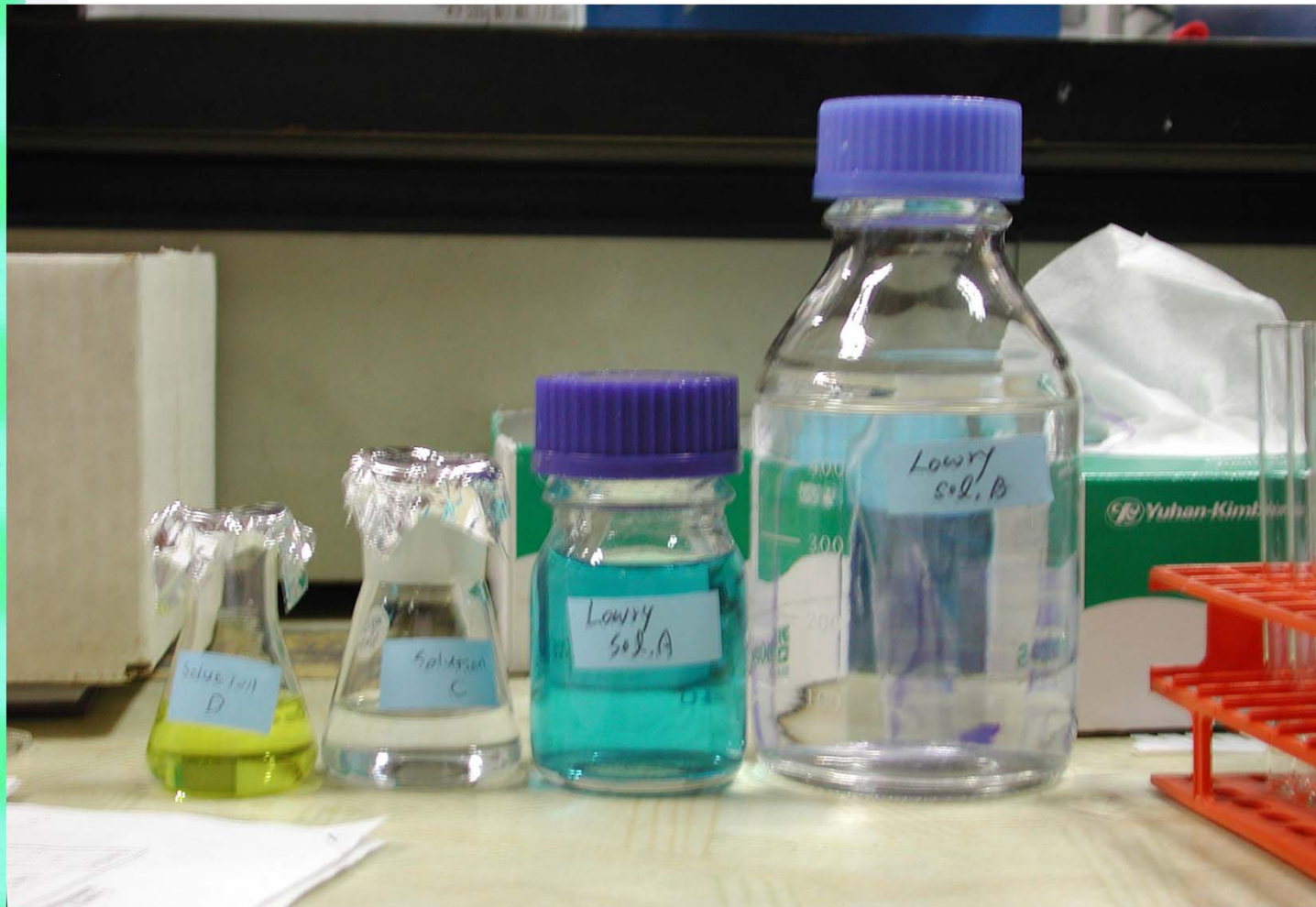


Fig. 1. Reagents for experiment.

1. Bring sample solution to 0.5ml with distilled water.



Fig. 2. 증류수 주입.

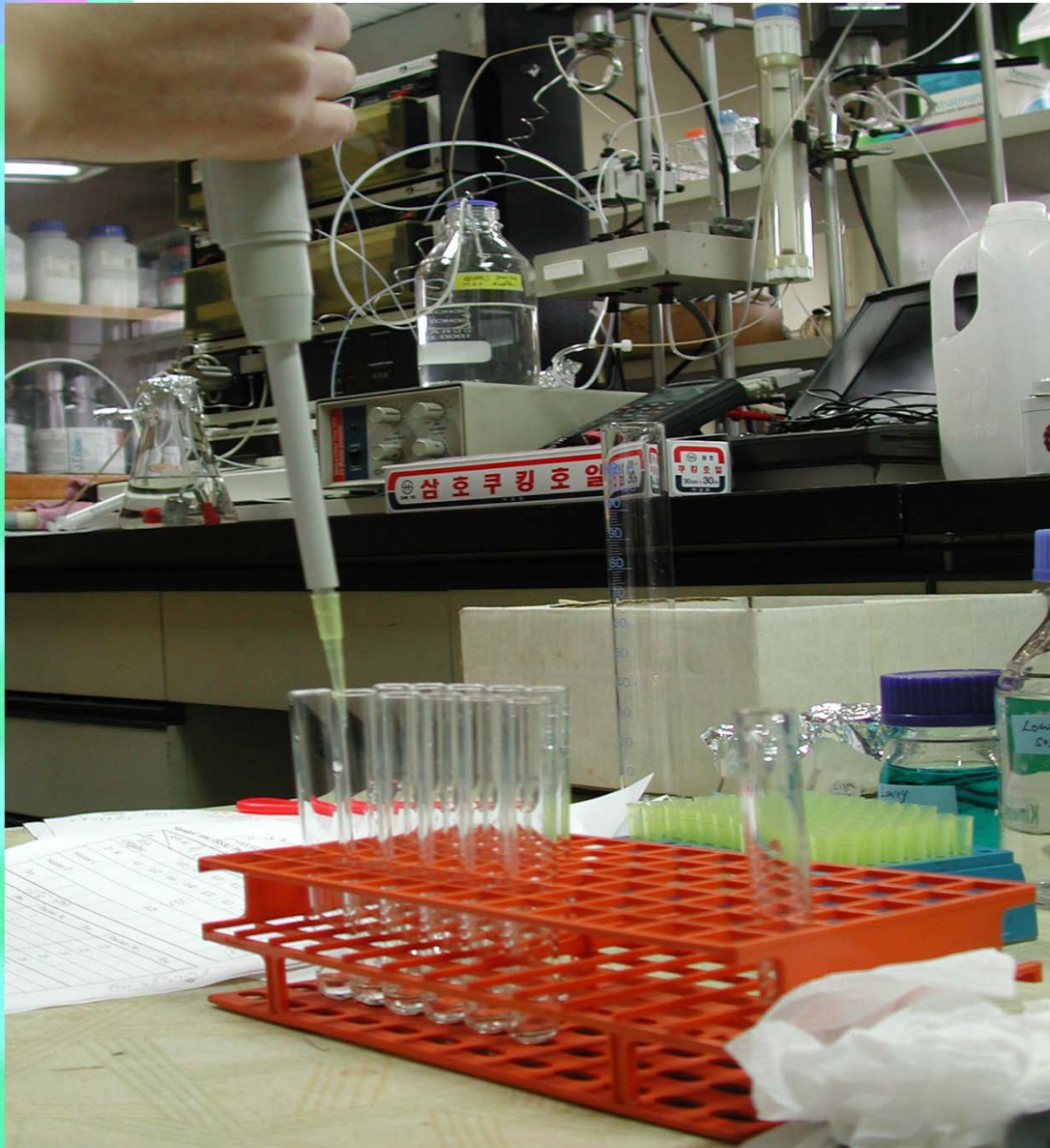


Fig. 3.
BSA 및 sample
주입.

2. Add 2.5ml Solution C.

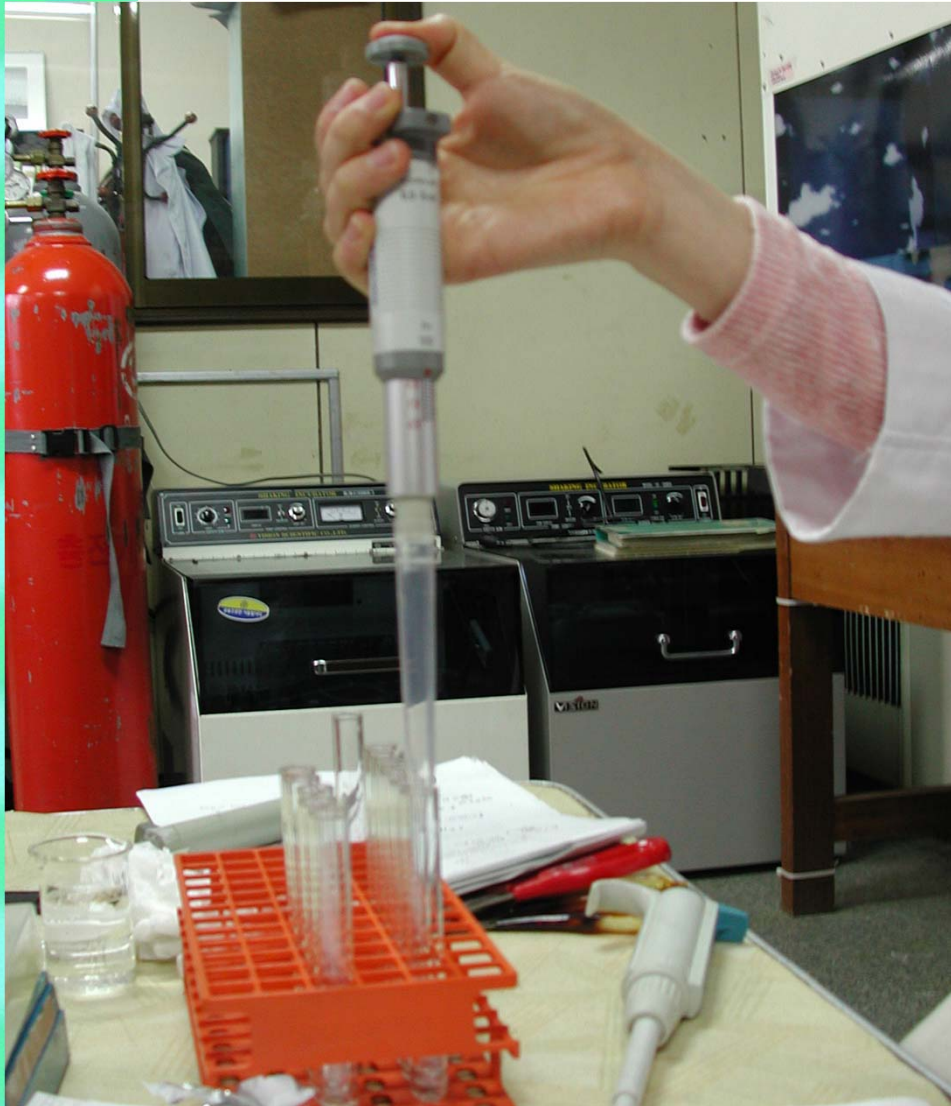


Fig. 4.

Solution C 주입.

3. Vortex and let stand at room temperature for 5~10minutes.



Fig. 5. Solution C를 넣은 후 교반.

4. Add 0.25ml Solution D and vortex.

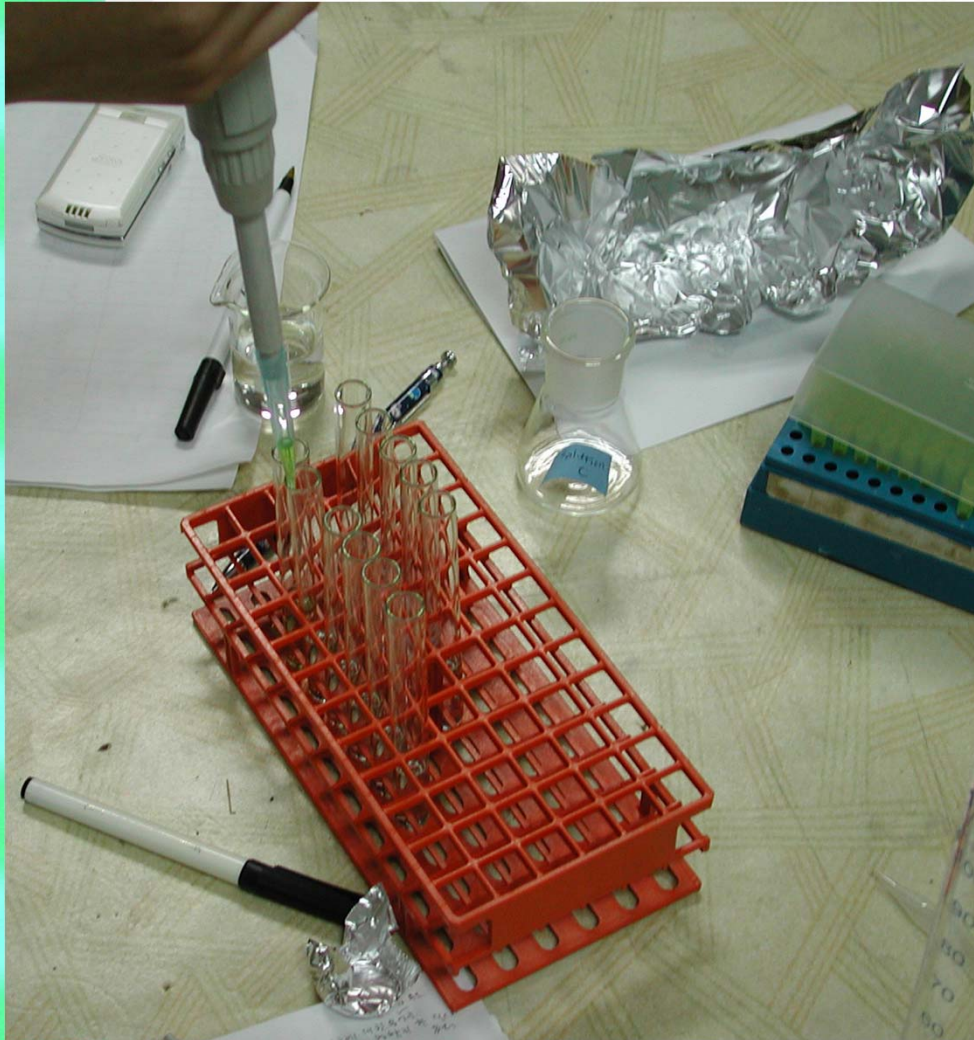


Fig. 6.

Solution D 주입.



Fig. 7. Solution D를 넣은 후 교반.

5. After 20~30 minutes, read A_{750} .

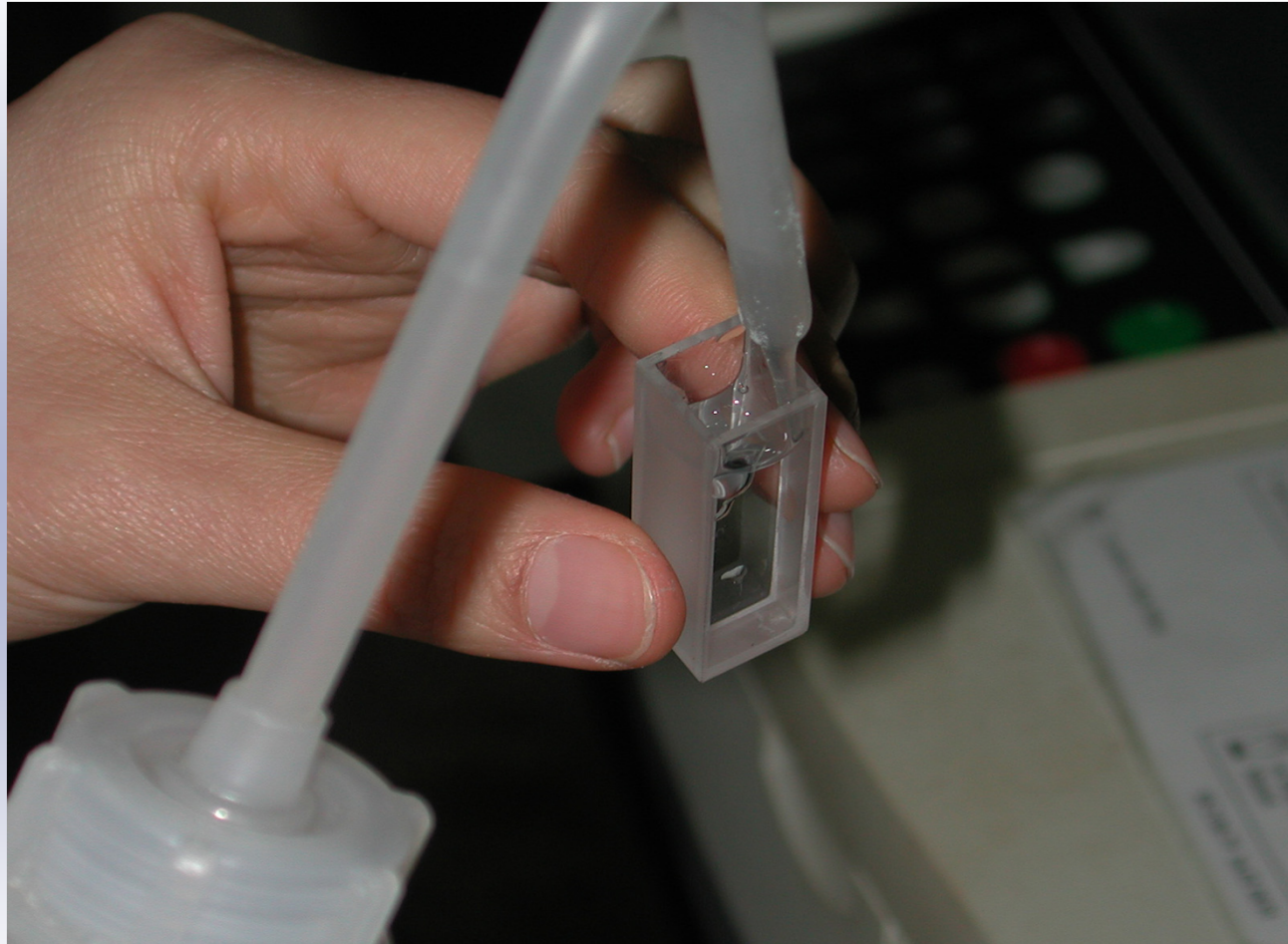


Fig. 8. 증류수로 cuvette 세척.*



Fig. 9. sample을 cuvette에 담기.



Fig. 10. spectrophotometer로 sample 측정.

Results

➤ A750에서 측정된 Standard의 값

Table 3. Standard 측정값.

Conc. ($\mu\text{g/ml}$)	A_{750}	Real A_{750}
0	0.063	0
4.5	0.070	0.007
9	0.125	0.062
30	0.171	0.108
60	0.292	0.229

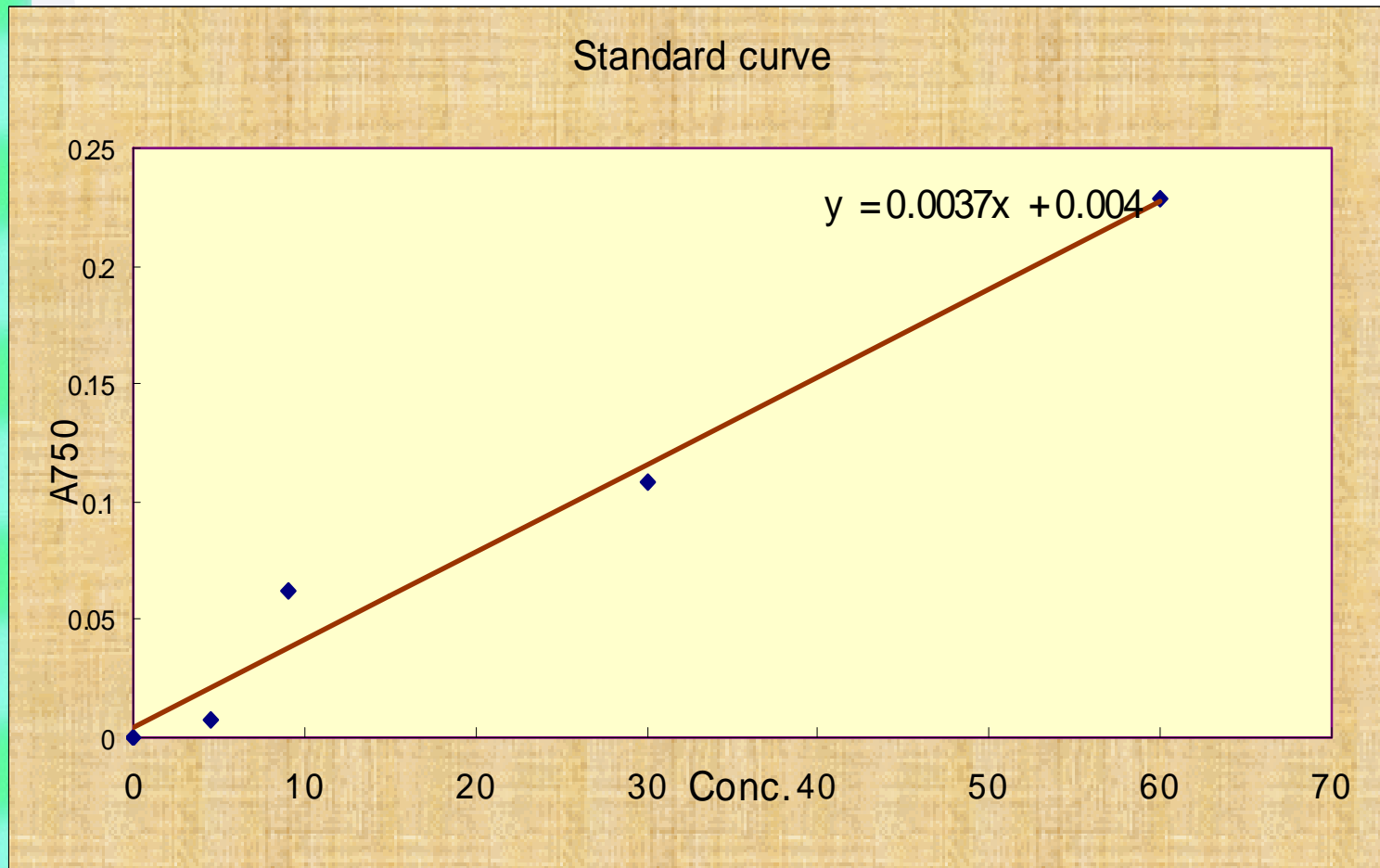


Fig. 11. Standard Curve.



➤ Sample의 측정값과 농도 계산

Table 4. Sample의 측정값과 농도.

No.	A_{750}	Real A_{750}	Conc.
1	0.436	0.373	99.73
2	1.134	1.071	288.38
3	0.63	0.567	152.16
4	0.6	0.537	144.05
5	0.263	0.2	52.97



➤ Sample의 단백질 계산

Table 5. Sample의 단백질 양.

No.	단백질의 양(μg)
1	3241.22
2	9372.30
3	4945.27
4	4681.76
5	1721.62