

himac

APPLICATION

Nov. 2005

Concentration of protein solution by means of ultrafiltration tubes

CF16RX/12RX/9RX versatile compact centrifuges

T6AP31 fixed angle rotor can concentrate a serum sample into the one about **ten times** thicker than before in a mere **ten minutes**.

Use of ultrafiltration tubes is becoming popular for concentration and demineralization of protein solutions. We developed T6AP31 fixed angle rotor (for culture tubes) for use with ultrafiltration tubes at the maximum speed 6,000 rpm (maximum RCF 4,770 x g). This rotor can quickly accelerate and decelerate thanks to its lightweight body made of resin (70% lighter than our conventional rotor). Following is our experiment result on concentrating protein sample in a standard serum by means of Millipore's Amicon® Ultra-4.

1. Experimental conditions

Centrifuge: himac CF16RX versatile compact centrifuge

Rotor: T6AP31 fixed angle rotor (for culture tubes)

Adapter: 15TA50T adapter

Speed: 6,000 rpm (maximum RCF: 4,630 x g (when using the above adapter))

Time: 10 minutes

Temperature: 15

Ultrafiltration tubes: Millipore's Amicon® Ultra-4 (30K) (molecular weight cutoff: 30,000)

Sample: Standard serum (Wako Pure Chemical Industries, Ltd.) diluted with ten parts of purified water

Amount of sample before concentrating: 3 ml

Amount of sample after concentrating: About 300 μ l

2. Protein assay reagent

"Protein Assay" by Bio-Rad

3. Result of experiment

(1) Calibration curve of protein concentration

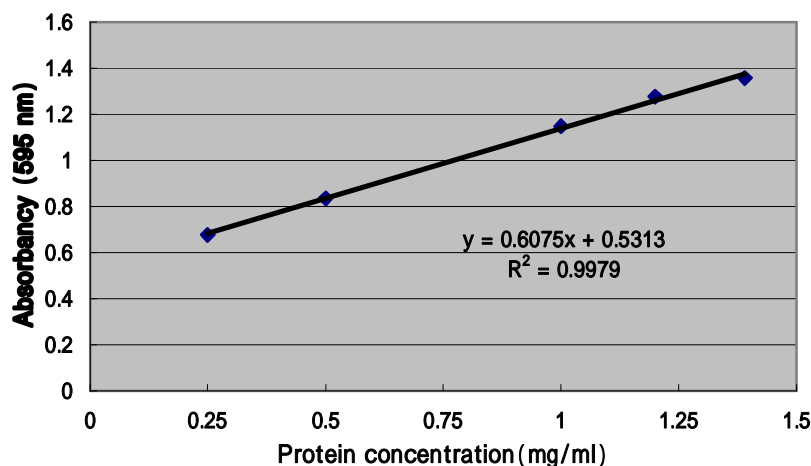


Fig. 2 Calibration curve of protein concentration (Sample: IgG)



Fig.1: T6AP31 fixed angle rotor

(2) Measured concentration before and after concentrating

Table 1. Result of measurement

Sample No.	Sample before concentrating			Sample after concentrating		
	Amount (ml)	Absorbancy (595 nm)	Concentration (mg/ml)	Amount (ml)	Absorbancy (595 nm)	Concentration (mg/ml)
Sample 1	3.0	1.072	8.900	0.3	1.157	103.0
Sample 2	3.0	1.140	10.02	0.3	1.144	100.9
Average			9.460			102.0

(Remark)

- (1) The sample before concentrating was diluted with ten parts of purified water then measurement was carried out.
- (2) The sample after concentrating was diluted with one hundred parts of purified water then measurement was carried out.

4. Experiment procedure

- (1) The standard serum dissolved in purified water was diluted with ten parts of purified water to make a protein solution of about 10 mg/ml. This solution was used as the sample.
- (2) 3 ml of the above sample were injected into Millipore's ultrafiltration tubes "Amicon® Ultra-4 (30K)" (molecular weight cutoff 30,000) respectively.
- (3) T6AP31 fixed angle rotor was loaded with 15TA50T adapter (standard equipment) and the above ultrafiltration tubes were set in the rotor.
- (4) The centrifuge was operated under the following setting conditions: 6,000 rpm, 10 minutes, 15 , maximum acceleration and deceleration levels.
- (5) The concentrated samples were collected and the protein concentration was measured.

5. Conclusion

T6AP31 fixed angle rotor could concentrate the sample of protein solution into the one about ten times thicker than before in a mere ten minutes as shown in Table1.

T6AP31 fixed angle rotor can also be used with CF5RX versatile compact centrifuge. In this case, however, the maximum speed is limited to 5,000 rpm (maximum RCF 3,310 x g (when using 15TA50T adapter)).

In addition to the adapter for Millipore's "Amicon® Ultra-4", the adapters for other ultrafiltration tubes such as Centricon, Centriplus and Ultrafree-CL are optionally available. Please select a proper adapter that is applicable to your ultrafiltration tubes.

For more information, visit our website at:

<https://ccs.hitachi-koki.co.jp/cgi-bin/himac/contactus/toiawase.cgi>

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