

PREPARATION OF WORKING PROTEIN STANDARD FOR USE IN PROTEIN DETERMINATION

CONDITIONS: T = 25°C, A_{280nm}, Light path = 1 cm

METHOD: Spectrophotometric Concentration Determination

REAGENTS:

- A. 0.85% (w/v) Sodium Chloride Solution (NaCl)
(Use Sigma Stock No. 430AG-4 or prepare 100 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)
- B. 0.5% (w/v) Working Protein Standard (WPS)
(Prepare 50 ml in Reagent A using Albumin Bovine Serum, Sigma Prod. No. A-4503 or Protein Standards, Bovine Serum Albumin, Sigma Prod. No. P-6529.)

PROCEDURE:

Pipette (in milliliter) the following reagents into a suitable cuvette:

	<u>Test</u>
Reagent A (NaCl)	0.90

Equilibrate to 25°C using a suitably thermostatted spectrophotometer. Monitor the A_{280nm} until constant. Record the Initial A_{280nm} for the Test. Then add:

Reagent B (WPS)	0.10
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Mix by inversion. Monitor the increase in A_{280nm} until constant. Record the Final A_{280nm} for the Test.

CALCULATIONS:

$$\text{mg protein/ml of WPS} = \frac{(\text{Final } A_{280\text{nm}} \text{ Test} - \text{Initial } A_{280\text{nm}} \text{ Test})}{(0.667)(0.1)}$$

WPS = Working Protein Standard

0.667 = Extinction coefficient of a 0.1% (w/v) BSA Solution at 280 nm

0.1 = Volume of Working Protein Standard used

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.