PROTEIN DETERMINATION
Biuret Method using TCA Precipitation

CONDITIONS:  T = 25°C, A_{540nm}, Light path = 1 cm

METHOD:  Colorimetric

REAGENTS:

A. 0.85% Sodium Chloride Solution (NaCl)
   (Use Stock No. 430AG-4 or prepare 100 ml in deionized water using Sodium Chloride, Prod. No. S-9625.)

B. 1% Working Protein Standard (WPS)
   (Use WPS prepared as per Working Protein Standard Procedure.)

C. Biuret Reagent (Biu)
   (Use Stock No. 540-2.)

D. Trichloroacetic Acid (TCA)
   (Use Stock No. 490-10)

E. Protein Sample Solution (Pro)
   (Prepare a solution containing 1 - 3 mg protein/ml of Sample in Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>----</td>
<td>0.98</td>
<td>0.95</td>
<td>0.90</td>
<td>0.70</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>B</td>
<td>----</td>
<td>0.02</td>
<td>0.05</td>
<td>0.10</td>
<td>0.30</td>
<td>0.50</td>
<td>----</td>
</tr>
<tr>
<td>E</td>
<td>1.00</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

Mix. Then add:

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>
PROTEIN DETERMINATION
Biuret Method using TCA Precipitation

Mix thoroughly and place the containers in an ice bath for 10 minutes. Centrifuge for 20 minutes in a clinical centrifuge. Remove and discard the supernatant. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (NaCl)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix thoroughly until pellet dissolves. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Biu)</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Mix thoroughly and seal the containers. Incubate for 30 minutes at 25°C. Transfer to suitable cuvettes and record the absorbance at 540 nm for Test, Standards, and Blank.

CALCULATIONS:

\[ \text{A}_{540\text{nm}} \text{ Standard} = \text{A}_{540\text{nm}} \text{ Std} - \text{A}_{540\text{nm}} \text{ Blank} \]

Find the slope (M) by plotting the \( \text{A}_{540\text{nm}} \) Standards vs Protein concentration.

Sample Determination:

Determine the concentration of protein using the following equation:

\[ \text{mg Protein} = \frac{\text{A}_{540\text{nm}} \text{ Test} - \text{A}_{540\text{nm}} \text{ Blank}}{(M)} \]

\[ \% \text{ Protein} = \frac{\text{(mg Protein)} \times (100)}{(\text{mg solid/ml Reagent D})} \]

For Products that are liquid:

\[ \text{mg Protein/ml} = \frac{\text{(mg Protein)} \times \text{(Dilution)}}{(\text{ml Reagent D})} \]

100 = Conversion to percentage
PROTEIN DETERMINATION
Biuret Method using TCA Precipitation

REFERENCES:


NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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