Bradford Reagent

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Product Code</th>
<th>Kit Packing</th>
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<tbody>
<tr>
<td>Bradford Reagent</td>
<td>ML106-100ML</td>
<td>100 ml</td>
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<tr>
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<td>ML106-500 ML</td>
<td>500 ml</td>
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Introduction: Bradford Reagent is formulated for rapid and accurate quantitative estimation of protein samples by following the Bradford assay method which was developed by Marion M. Bradford in 1976. This reagent is conveniently supplied as ready-to-use form and no dilution is required while performing the assay.

Description: Bradford Reagent contains a dye, Coomassie brilliant Blue G-250, which has an absorbance maximum of 465 nm in unbound state. The Bradford protein assay is based upon the formation of complexes between Coomassie Brilliant Blue G-250 and the protein samples in solution. When the protein sample binds to the dye the color of the solution turns blue from brown and there is a shift in the absorption maximum of the dye from 465 nm to 595 nm. This dye binding procedure is completed within 5 minutes and the blue colored complex formed is stable for one hour. Thus concentration of unknown protein sample can be derived by plotting its absorbance value on the standard curve. The standard curve is obtained from the absorbance readings of the series of standard protein dilutions assayed along side the unknown sample.

Application: Bradford reagent is mainly used for the quantitative estimation of protein samples by the Bradford assay method.

Composition: Bradford reagent consists of Coomassie Brilliant Blue G-250 in phosphoric acid and ethanol.

Properties:
- Appearance: Brown colored solution
- Clarity: Clear and free of particles
- Suitability test: This solution has been tested and is suitable for use in quantitative estimation of protein samples by Bradford assay

Storage conditions: Bradford Reagent has to be stored at 2 - 8 °C

Quality control: Bradford Reagent is tested for quantitative estimation of unknown protein samples.

Estimation of unknown protein using standard curve (with BSA)

1. Take seven test tubes and label them as Blank and 1 to 6.
2. Make dilutions of standard protein (BSA) with concentrations of 20, 16, 12, 8, 4 µg/200 µl by transferring respective amount of BSA solution (stock: 1mg/ml) and adjusting it to a total volume of 200 µl by adding distilled water as mentioned in the following table.
3. Add 1ml of Bradford’s Reagent to each test tube and mix the contents of each tube thoroughly by vortexing the tubes and incubate at room temperature (below 30°C) for 10 minutes.
4. Transfer the content of the tubes to cuvettes and measure the absorbance at 595 nm wavelength.
5. Plot a Standard Curve of absorbance at 595 nm on “Y” axis versus concentration of protein µg/200 µl on “X” axis.

6. Record the value “x” of unknown sample from graph corresponding to the absorbance reading.

**Fig: Standard curve for protein estimation by Bradford assay**

Protein concentration can be calculated using following formula:

\[
\text{Protein Concentration in Unknown Sample} = \frac{\text{Concentration of Unknown in “µg”}}{\text{Volume of sample in “µl”}} \times 1000 \text{ µg/ml}
\]

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