

Bradford Protein Assay

Set up of Standards

1. Set up standards in duplicate in 13mm disposable test tubes.
2. The total volume in each tube should always equal 100 μ L.
3. BSA is used as the protein standard.

Example

The following is an example of the set-up of standards. For this example the standard had a concentration of 0.212 μ g/L. The tube you are using

should have the concentration of the BSA written on it. BSA is stored @-20C, on the freezer door.

BSA (μ L)	ddH ₂ O (μ L)	Concentration (μ g)
0	100	0
10	90	2.12
20	80	4.14
30	70	6.36
40	60	8.48
50	50	10.6
60	40	12.72
70	30	14.84

1. Add 1.0mL diluted (1:5) Bradford Reagent per tube. Vortex.
2. Incubate at room temperature for 5 minutes.
3. Spec. each tube at multi-wavelength A595. (Sample extracts should be spec'd. right after standards.)

Set-up of Sample Extracts

1. Sample extracts should be done in duplicate in 13mm disposable test tubes.
2. 100 μ L of each sample extract is placed in test tube. If a dilution of the extract needs to be made, then ddH₂O should be added so that the

- final volume is still 100 μ L in each test tube.
3. Add 1.0mL diluted Bradford Reagent per tube. Vortex.
 4. Incubate at room temperature for 5 minutes.
 5. Spec. each tube at multi-wavelength A595. The standards and the sample extracts should be set-up at the same time so that the sample extracts can be specd. right after the standard.

Analysis of Samples

1. Set spectrophotometer on Multi-wavelength A595.
2. Calibrate spec. on ddH₂O.
3. Read all standards then all sample extracts.

Calculations

$$\text{mg/mL} = \frac{\text{mean in g} \times 1.0\text{mg} \times 1000\text{L}}{\text{sample amt. in L}}$$

$$\text{sample amt. in L} = \frac{1000\text{g}}{1.0\text{mL}}$$

Solutions

1. Dilute Bradford Reagent (1:5 dilution)

Make a 1:5 dilution of Bradford protein dye to ddH₂O.

EXAMPLE: 10mL Bradford Protein dye to 40mL ddH₂O

Concentrated Bradford Reagent is stored at 4°C.

Diluted Bradford Reagent should be made fresh, it should not be stored for longer than a week at 4°C.

2. BSA. A stock of BSA of about 1mg/mL should be made.

Aliquot 1mL per microcentrifuge tubes tube.

Stored at -20°C.

3. BSA Standard.

Take 2mL stock BSA 1mg/ml

8mL ddH₂O.

Spec. 1mL at A_{260/280}.

(A₂₈₀)x(1mg/mL) = prot mg/mL

(0.66)

Aliquot 700μL per tube.

Store at -20°C.