BioRad bovine serum albumin (BSA) Protocol

https://www.bio-rad.com/webroot/web/pdf/lsr/literature/4110065A.pdf

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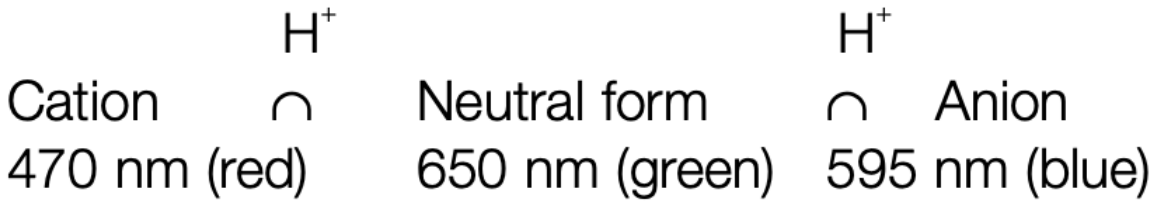
Introduction: The Bradford assay is a protein determination method that involves the binding of Coomassie 1 4110065A.qxp Brilliant Blue G-250 dye to proteins (Bradford 1976). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (Amax = 470 nm). However, when the dye binds to protein, it is converted to a stable unprotonated blue form (Amax = 595 nm). Beer's law may be applied for accurate quantification of protein by selecting an appropriate ratio of dye volume to sample concentration.



There are certain known sources of contamination that will shift the color of the blue dye, such as some detergents, flavonoids, and basic protein buffers, stabilize the green neutral dye species by direct binding or by shifting the pH (Compton and Jones 1985, Fanger 1987). Therefore, the BSA standard would be an appropriate standard if the sample contains primarily albumin, or if the protein being assayed gives a similar response to the dye. For a color response that is typical of many proteins, the gamma-globulin standard is appropriate. For our assay, we should use both the first time to double check.

Microplate Assay Materials: 250 µl microplate assay. The linear range of these assays for BSA is 125–1,000 µg/ml, whereas with gammaglobulin the linear range is 125–1,500 µg/m

- [ ] 300 uL microplate
- [ ] 595 nm filter and spec (one read only)
- [ ] Microplate mixer
- [ ] Multi-channeled pipette (250uL)
- [ ] P10 and p1000
- [ ] Dye reagent (from kit)
- [ ] Buffer from CS kit
- [ ] Razor blade

Procedure:

1. Remove the 1L dye reagent from the 4°C storage and let it warm to ambient temperature. Invert the 1L dye reagent a few times before use
2. Weigh tissue (< 20 ug/sample), refreeze leftover tissue for duplicates (relabel tube)
3. Take out frozen oyster samples and homogenize tissue with (350uL) CS buffer in 2mL tubes using **p1000 eppendorf**.
4. Centrifuge oyster tissue and collect supernatant (~350uL)
5. Vortex supernatant and spin down again
6. Dilute protein standard (BSA and gamma-globulin) in triplicate. See dilution table for volumes. For the diluent, use the same buffer as in the samples. Need to make fresh sample curve for each day (not each sample)
7. Make blanks
8. Proportion dye into well plates- **250 µl per well** - using multichannel p250 and 300uL pipette tips (could also use **p200** maybe) Be careful not to make bubbles, spin down with a plate spinner at room temperature if bubbles show. (p200 x 12 per plate)
9. Proportion of dye to sample protein or to standard is 5:0.1ml (or 50:1ml) (or 50,000:1uL), add dye to protein and mix well (with multichannel pipette) Microplate **5 µl (standard and sample)** using **p10**, do whole plate first
10. Incubate at room temp for 5 min (no longer than 1 hour)
11. Measured at ~595nm (570nm) absorbance and record
12. Create a standard curve by plotting the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis). Determine the unknown sample concentration using the standard curve.

Microplate Standard Assay

Tube #	Standard Volume (µl)	Source of Standard	Diluent Volume (µl)	Final [Protein] (µg/ml)
1	20	2 mg/ml stock	0	2,000
2	30	2 mg/ml stock	10	1,500
3	20	2 mg/ml stock	20	1,000
4	20	Tube 2	20	750
5	20	Tube 3	20	500
6	20	Tube 5	20	250
7	20	Tube 6	20	125
8 (blank)	–	–	20	0

Tube #	1st Reagent	Volume Added	2nd Reagent	Volume added	Final V (3 reps each)
1	BSA standard	20 uL	-		20 uL
2	BSA standard	30 uL	buffer	10 uL	40 uL (-20 uL)
3	BSA standard	20 uL	buffer	20 uL	40 uL (-20 uL)
4	Tube #2	20 uL	buffer	20 uL	40 uL
5	Tube #3	20 uL	buffer	20 uL	40 uL (-20 uL)
6	Tube #5	20 uL	buffer	20 uL	40 uL (-20 uL)
7	Tube #6	20 uL	buffer	20 uL	40 uL
8	buffer	20 uL	-		20 uL

BSA standard curve I've actually used. ug/mL is protein output from BSA kit, A is absorbance