BIO-RAD BRADFORD TOTAL PROTEIN ASSAY

(Bradford, 1976 & Wright *et al.*, 1996)

Introduction

This procedure is used to measure protein concentration in samples that were extracted for glomalin, using either the total or easily extractable extraction procedure. This assay does not give the most accurate glomalin concentration, because it is not specific for glomalin, but it will measure any protein that has survived extraction. Total protein values are usually higher than the values calculated from the ELISA procedure, but running this assay will give an estimate of glomalin concentration and help determine the volume needed for the ELISA.

Materials

PBS (phosphate buffered saline), pH 7.4
BSA (bovine serum albumin)
Bio-Rad protein dye
96-well microtiter plates or ELISA strips
Micro-pipetter and tips
Dissecting needle
Plate reader (see below), with 590 or 595nm filter

Methods

1) Prepare standard curve, using BSA.

BSA standard curve preparation

A. Make 1 ml stock solutions of 10 ug BSA/200ul PBS (10 mg/200 ml) and freeze, until needed.
B. Thaw and dilute with PBS as outlined below:

BSA standards for Bradford protein assay			
Well designation	ug/well	BSA stock solution(ul)	PBS (ul)
Blank	0	0	200
Standard 1	0	0	200
Standard 2	1.25	25	125
Standard 3	2.5	50	150
Standard 4	3.75	75	125
Standard 5	5	100	100

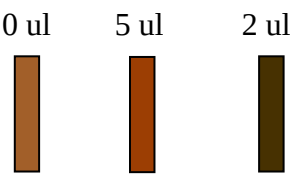
2) Add 200 ul of PBS minus the volume of extract to each well. (For example, if there is 5 ul of sample in the well, 195 ul of PBS is needed.) Extract volume will typically be 10 ul, 5 ul, or 2 ul depending on color. (Follow color chart to determine amount of sample to add).

Color chart for sample volume determination

A. The color of the extract can help determine the right amount of sample to use for the reading to be somewhere within the values of the standard curve.

Sample color	ul sample/well
Golden	50+
Golden-brown	25-50
Brown	10-25
Reddish brown	5-10
Reddish black	1-5

Example of colors of extracts and amount of sample needed



3) Start 5 min on timer. Carefully add 50 ul of Bio-Rad protein dye to each well (Fig. 1). **Mix thoroughly with pipette.** (Note: Because this reaction happens so rapidly and it takes time to pop the bubbles, it is advised to only add dye to 4 rows or strips at a time, but the same curve can be used for all 8 rows.)

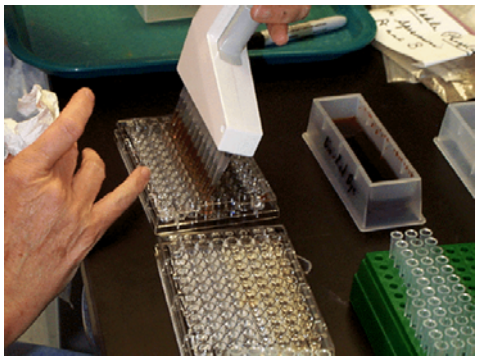


Figure 1. Bio-Rad protein dye is added to each well.

4) Pop bubbles with dissecting needle (cleaning needle between samples) and read after the 5 min has expired.
5) Use BSA standard curve to calculate mg protein/g material extracted (see below).

OD value range for the total protein standard curve

Older dye, typical		Newer dye, recent values	
Concentration (ug/well)	OD value	Concentration (ug/well)	OD value
0	0	0	0
1.25	0.100-0.200	1.25	0.175-0.225
2.5	0.200-0.300	2.5	0.325-0.400
3.75	0.300-0.400	3.75	0.450-0.550
5.0	0.400-0.550	5.0	0.600-0.700

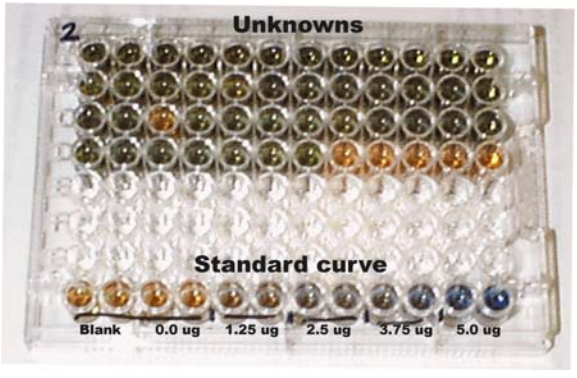


Figure 2. Example of plate after dye has been added and mixed – red indicates no protein while the intensity of blue is related to concentration of protein.

Calculating mg/g concentration from OD values

A. After running the assay, use the standard curve to determine the concentration according to OD values (protein concentration in a well of between 0-5 ug).
B. Take this number and divide it by the number of ul of sample that in the well, giving a ug/ul value.
C. Then multiply this number by the number of ul extracted (the volume of the extract solution), resulting in an ug extracted value.
D. Finally, divide this number by g weight extracted and resulting in a ug/g value that can be converted in a mg/g value by dividing by 1000.

Example:

OD reading = 0.306
ug/well concentration (from OD value and standard curve) = 2.54 ug/well
ul of sample/well = 10 ul/well
amount of extract = 7650 ul (7.65 ml)
weight extracted = 1.0 g

2.54 ug/well ÷ 10 ul/well x 7650 ul ÷ 1.0 g = 1943.1 ug/g = 1.9431 mg/g