Total protein measurement

This protocol is designed to measure total protein from fly homogenates prepared from triglyceride or carbohydrate measurements. Samples should be kept cold prior to this analysis.

1) Prepare a series of BSA standard stock solutions. Most restriction enzymes from NEB are supplied with a 10 mg/ml BSA stock, which can be used for this purpose. Also, BSA concentration can be verified using a spectrophotometer (A_{280}=0.667 for 1 mg/ml BSA). Generate a 0.5 mg/ml standard by diluting 5 µl of 10 mg/ml BSA with 95 µl of PBS (100 µl final volume). Conduct a series of 2-fold serial dilutions into PBS (e.g. 50 µl of 0.5 mg/ml diluted in 50 µl PBS for a 0.25 mg/ml standard) to generate a 0.0625, 0.125, 0.25 and 0.5 mg/ml protein standards. Aliquots of BSA stock solution can be stored at -20ºC.

2) Dilute the 10 µl of homogenized sample between 1:10 to 1:20 in cold PBS. Centrifuge the samples at maximum speed for 5 minutes in a tabletop centrifuge to remove any precipitate.

3) Pipette 10 µl of a PBS blank and the BSA dilution series into the first row of a clear-bottom 96 well plate. Transfer 10 µl of supernatant from each homogenized fly sample into individual wells of the subsequent rows.

4) Add 200 µl of Bio-Rad Protein Assay (diluted 1:5 with water) to each sample using a multichannel pipette.

5) Incubate the plate at room temperature for 5 min. Centrifuge the plate in an appropriate swing-bucket rotor to remove any air bubbles present in the samples. Use a plate reader to measure absorbance at 595 nm.

6) Determine the protein concentration for each sample based on the BSA standard curve.