This procedure is used to measure the protein content of samples derived from cell lines and primary cell cultures. The use of trichloroacetic acid (TCA) to precipitate proteins permits removal by filtration of interfering substances in the various sample preparations; these include detergents, dyes, and reducing agents. The procedure described in this protocol is modified from Schaffner and Weissman, *Anal Biochem.* 56, 502-514 (1973).

**Determination of Protein Content in Standards and Samples**

1. Prepare samples for a standard curve by pipetting 0, 5, 10, 20, 40, and 80µl of bovine serum albumin (fraction V), 0.2 mg/ml (0.2 mg/ml BSA) into 1.5-ml microfuge tubes or 10 x 75 mm disposable glass culture tubes; this provides known protein amounts of 0, 1, 2, 4, 8, and 16µg. Adjust the total volume of each well to 225 µl with purified water.

2. Pipette aliquots of unknown samples into individual tubes (estimated to contain 1 to 15 µg of protein). Adjust the total volume of each sample to 225 µl with purified water. (For large numbers of samples, standards and sample aliquots can be added to and mixed in individual wells of 96-well non-tissue culture plates and processed similarly.)

3. Add 30 µl of Tris-HCl, pH 7.5, 1 M with sodium dodecyl sulfate (SDS) 2% (Tris-HCl/SDS) to each sample. (Since the solution contains SDS, care should be taken to avoid formation and pipetting of bubbles, which can reduce the accuracy of additions.)

4. Add 50 µl of 90% trichloroacetic acid (90% TCA) to each well.

5. Vortex the samples. (For 96-well plates, use a repeat pipettor, set at <200 µl, to pipette samples up and down; change tips between rows.)

6. Incubate all samples for at least 2 min at room temperature to precipitate protein.

7. Transfer samples into wells of the Bio-Dot manifold (see AfCS Protocol Manifold Assembly—Bio-Rad Bio-Dot). Make sure pipette tips do not come into contact with the membrane. Leave each used tip in the appropriate tube (or well of a 96-well plate).

8. Apply gentle vacuum to drain the wells.

9. Rinse the sample tubes (or wells) with 200 µl of 6% trichloroacetic acid (6% TCA). Transfer the rinse to the appropriate manifold well, using the same tip used to load the protein sample in step seven. At this time, both the tube and the tip for each sample may be discarded.

10. Apply gentle vacuum to drain the wells.

11. Add 100 µl of 6% TCA to each well, and apply vacuum to drain the wells. Be sure the solution in each well is drained thoroughly. For samples with a high protein content, this may take 5 min or longer.

12. Unscrew the manifold and use forceps to carefully remove the membrane. Wear gloves from this point on, or use blunt forceps. Cut out the section of membrane containing filtrate from the wells that were used for samples.

13. Place the piece of membrane containing filtrate in a suitable container (e.g., a small plastic box or glass dish).
14. Add a minimal volume of Amido black stain solution (Amido black stain) to cover the filter.

15. Stain the membrane for 20 to 45 min. Pour off the stain solution into a container for used stain (the stain solution can be reused 2 to 3 times).

16. Rinse the stained membrane for 30 to 45 sec with water; pour off the water.

17. Destain the membrane with three successive rinses with Amido black destain solution (Amido black destain). Each rinse should incubate for 2 to 3 min (with gentle shaking) before transfer of the membrane to the next rinse. The rinse solutions are kept in separate containers and can be reused 2 to 3 times, with the last rinse always being fresh Amido black destain.

18. Rinse membrane in water for 2 to 3 min with gentle shaking (membrane should sink when the organic solvent is removed).


20. Label a set of fresh microfuge tubes or 10 x 75 mm glass tubes appropriately.

21. Carefully excise the stained spots with a single-hole paper punch or razor blade. The excised pieces containing stained filtrate (protein) should be of similar size.

22. Place the excised sample in individual new tubes.

23. Add an equal volume (0.25 to 0.5 ml depending on requirements for reading optical density [OD]) of Amido black elution solution (elution solution) to each tube. Let the stain elute for 10 min or more with occasional vortexing. The eluted stain is stable for less than two hr.

24. Measure the absorbance of each eluate at 630 nm. The spectrophotometer is zeroed against the elution solution.

25. If a 96-well plate reader is available, transfer 200 µl of each eluted sample into appropriate wells of a 96-well plate and read absorbance of wells at 630 nm.

Calculations

26. A standard curve is generated by plotting the OD of the protein standards versus their content of protein (as µg, ranging from 0 to 16). The data are fit by linear regression analysis.

27. The protein concentration (µg/µl) in unknown samples is calculated from the standard curve and the volume of samples used in the assay.

Reagents and Materials

Bovine serum albumin (fraction V), 0.2 mg/ml (0.2 mg/ml BSA): AfCS Solution Protocol ID PS00000018

Microfuge tubes, 1.5 ml: Eppendorf; catalog no. 2236320-4

Disposable glass culture tubes, 10 x 75 mm: Fisher Scientific; catalog no. 14-961-25

96-well, flat-bottom microtiter plate (non-tissue culture): Dynex Technologies; catalog no. 3855

Tris(hydroxymethyl)aminomethane-HCl, pH 7.5, 1 M with sodium dodecyl sulfate, 2% (Tris-HCl/SDS): AfCS Solution Protocol ID PS00000068
Trichloroacetic acid, 90% (90% TCA): AfCS Solution Protocol ID PS00000013

Repeat pipettor: Eppendorf; catalog no. 2226020-1

Bio-Dot Apparatus: Bio-Rad; catalog no. 170-6545
   Includes Bio-Dot sample template; vacuum manifold base; gasket support plate; and gasket
Trichloroacetic acid, 6% (6% TCA): AfCS Solution Protocol ID PS00000010

Amido black stain solution (Amido black stain): AfCS Solution Protocol ID PS00000015

Amido black destain solution (Amido black destain): AfCS Solution Protocol ID PS00000014

Amido black elution solution (Elution solution): AfCS Solution Protocol ID PS00000016

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