AMIDO BLACK PROTEIN ASSAY

jwbrown@crab Wed Jan 15 02:45:08 1992
Subject: Micro-protein assay

This micro-protein assay is not as accurate as other methods for assaying protein concentration, but it has the distinct advantage of requiring only trace amounts (less than 1ug) of your valuable protein. In many cases the accuracy (within a factor of 2) of the assay is more than sufficient. Another advantage of the assay is its relative ease and quickness.

Amido black stain

0.25% Amido black	0.625g
45% MeOH	112.5ml
45% ddH2O	112.5ml
10% glacial HOAc	25ml

Amido black destain

same as the stain without the dye

Cellulose acetate dissolving solution

80ml formc acid
10ml glacial HOAc
1ml 100% TCA

Starting with 5mg/ml BSA (in saline), make a series of 1:2 dilutions in saline (100ul BSA + 100ul saline). 8 - 10 dilutions is fine.

In duplicate, spot 1ul of each BSA dilution, and the samples to be tested to a strip of cellulose acetate.

Allow the spots to air-dry. If the samples contain alot of sucrose or glycerol. Re-wet the spot with 2ul aliquots of ddH2O & allow to dry again. Repeat this 3 - 5 times.

Stain in amido black stain for 10 minutes, then destain in several transfers of destain until the background is nearly white.

Examine the intensities of the black spots. Compare the spots from your test protein with those of the standards to obtain an estimate of your protein concentration.

If a more accurate estimate is required:

Carefully punch out one of each duplicate spot & dissolve in 400ul dissolving solution

Measure the A523 of each sample. Plot the BSA standards as a standard curve & interpolate the concentration of the test samples using this curve.

Michael Thomm, personal communication
JWBrown, unpublished data