Fluorescamine Assay to Determine Protein Content in Polymer Microparticles

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Reagents

Fluorescamine
Acetone
96 Well Plate (solvent resistant)

Procedure

1) Have samples in 150 ul buffer
2) In the first two wells of row A, place 300 ul of the standard
3) In the first two wells of the rest of the rows, add 150 ul of sodium acetate buffer
4) Do a 2 fold serial dilution down the column, excluding the last row
5) Add each sample in 150 ul quantity to two wells (duplicates)
6) Determine how much fluorescamine is needed by multiplying the number of wells x 50ul and then add approx. 300 ul extra
7) Make the fluorescamine solution in acetone in a 3 mg/ml concentration
8) Add 50 ul of fluorescamine solution to each well
9) Place film sticker over the plate
10) Take plate to plate reader and make sure it is on fluorescence
11) Go to excitation and emission. Excitation set at 400nm and Emission at 460nm
12) Go to sensitivity, reading should be 40 or above
13) Define the wells to read
14) Take plate and peel off film, then place in the drawer of reader
15) Click on Read, the drawer will close
16) Plate will read
17) Export file as needed.
18) Shut off machine, making sure drawer is closed
19) E-mail or save the file and come back to lab
20) Open file in Excel
21) Make a calibration curve of the standards with the average of the duplicates as the x-axis and the concentration as the y-axis
22) Obtain regression equation and analyze results