modified Bradford procedure for residual protein testing in enzyme catalysis synthesis products

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Abstract

Provide a modified Bradford method to determine the residual protein content in the bio-synthesis product.

The product may be obtained from enzymatic reaction and extract from organic solvent, and /or insoluble in water.

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Guidelines

May not suitable the product wo dissolved in some organic reagents/ water buffer, need to test.

Protocol

Step 1.
. Prepare the standard residual protein (like the standard purified enzyme who catalysis the product synthesis, or BSA). This may be need you to special purify the protein and determine the content of pure protein by common Bradford method or from SEC-HPLC. The standard protein should be carefully weighed.

Step 2.
The standard residual protein need to dissolve in the water buffer (e.g. the final content may be 1 mg/mL). The buffer could be the enzyme soluble buffer used for catalytic reaction. The make a series of standard solution of residual protein. The dilution buffer is the organic reagent (free of protein) that used to dissolve the final synthesis product with the aqueous solution be fully miscible. The dilution should also be accurate, preferably in the capacity bottle.Note: Over-concentrated samples will become viscous, and may not achieve proper extraction of protein. Samples must remain free flowing. Samples which are too concentrated can be diluted to an acceptable range by addition of organic reagent.

Step 3.
Standard Solution Extraction: The standard solutions are extracted with an organic:aqueous phase ratio of 2:1. Then 150 µL of each protein sample solution in organic reagent and 75 µL of aqueous phase like dH2O were charged to a 1.5 mL Eppendorf tube. Each solution was vortexed for at least 1 minute and centrifuged for 10 minutes at max krpm to achieve phase separation. The aqueous phase

was separated from the organic phase for assay via Bradford in triplicate.

Step 4.

Bradford Assay: The resulting aqueous phase protein concentrations after extraction should vary between 100-600 µg/mL. Then we could do protein content determination follow the common Bradford procedure. For example, each sample aqueous solution will blend with room temperature Commassie Plus Protein assay reagent (1:50). Then reactions will incubated for 10 to 60 minutes at room temperature. The resulting solution absorbance was measured at 595 nm using the spectrophotometer or other machines like Microplate Reader.The absorbance values of the standard solutions were averaged and normalized against the 0 µg/mL protein control. The resulting absorbance values were used to create a calibration curve containing NLT 6 evenly spaced points. The calibration curve should fit a linear regression with an Rsq value NLT 0.99 for protein aqueous solutions.