Hydrophobic Protein Analysis Kit (Product M0794)
Product Information Sheet (0794-008)

NOTE: The following information is given as a viable methodology for use of the Hydrophobic Protein Analysis Kit. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

I. OVERVIEW

Many native proteins contain varying ranges of hydrophobicity. Upon binding of these hydrophobic regions, TNS, a normally non-fluorescent compound in aqueous solution, exhibits a strong fluorescence enhancement. The degree of fluorescence enhancement is proportional to the protein hydrophobicity, and linear over a wide range of concentrations. The effects of detergents (e.g., Triton X-100, sodium dodecylsulfate) and other hydrophobic protein isolation buffer additives (e.g., sodium citrate, TRIS, sodium salicylate, L-cysteine, 2-mercaptoethanol, DMSO) that often interfere with standard protein assays are found to be minimal, due to the fact that the assay is primarily based on hydrophobic surface area content of the protein analytes. The combined effects of TNS stability, low fluorescence background, and high quantum yield enhance the utility and sensitivity of this assay. Moreover, TNS shows a high selectivity toward hydrophobic proteins, which may provide a direct tool to monitor these proteins in non-homogeneous protein samples and in SDS-PAGE systems.

The Hydrophobic Protein Analysis Kit provides a quick estimation and/or comparison of hydrophobic proteins. The Kit provides reagents and protocols for analyzing various proteins in their native and denatured conformations and for SDS-PAGE analysis. A total of 100 assays may be performed with this kit and up to three different protein samples can be analyzed.

II. MATERIALS

A.) **Buffer Solutions**. Buffer solutions A and B contain 20mM TRIS-HCl, pH 7.5, 150mM NaCl, and 5mM 2-mercaptoethanol. Buffer B also contains 0.1% SDS (sodium dodecyl sulfate) (w/v) as a denaturing reagent.

B.) **Reference Standards**. Reference standards A and B were prepared by dissolving purified BSA (bovine serum albumin) in buffers A and B, respectively, yielding a final protein concentration of 1mg/mL. Serial dilutions analyses of these reference standards should be performed concurrent with other protein samples, typically using the concentrations of: 500µg/mL, 250µg/mL, 125µg/mL and 62.5µg/mL.

C.) **Fluorescent Reagent A:** A solution of 1mM 6-p-Toluidino-2-Naphthalenesulfonic

acid (TNS, free acid) in 10%DMSO/H_2O, for use in the soluble assay described below.

D.) **Fluorescent Reagent B:** A 5mM solution 6-p-Toluidino-2-Naphthalenesulfonate Potassium salt (TNS K+) in H_2O for use in SDS-PAGE gels as described below.

D.) **Storage and Handling**. Fluorescent reagents, standards, and buffers should be handled with care, kept cold when not in use, and stored at 4ºC. In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition.

III. ASSAY CONDITIONS

To perform the assay on protein samples in their native conformations, dissolve samples in Buffer A and make serial dilutions with the same buffer to give concentrations of 1mg/mL, 500µg/mL, 250µg/mL 125µg/mL, and 62.5µg/mL. To run the assay on proteins in their denatured state, dissolve in Buffer B and perform the same dilutions in Buffer B as above. Co-assay with the BSA Reference Standard is recommended. To run the reference standard assay, perform serial dilutions of Standard A in Buffer A (native) and Standard B in Buffer B (denatured) to yield the same concentrations as that of the protein samples. To a 96-well microtiter plate well, add 100µL of fluorescent reagent **(A)** (1mM TNS (free acid)/10% DMSO) and 100µL of each protein sample or standard dilution in triplicate. For the control, add 100µL of fluorescent reagent **(A)** and 100µL of respective buffer. Best results are typically found 1-2 hours after the protein samples are introduced into the 1mM TNS/DMSO solution. Fluorescence readings can be measured with emission and excitation filters set at 465nm and 360nm, respectively. Data can be plotted as shown below in Figures 1 and 2. Calculated hydrophobic surface area data can be obtained at the following Web site: http://www.rcsb.org/pdb/ (The Protein Data Bank).

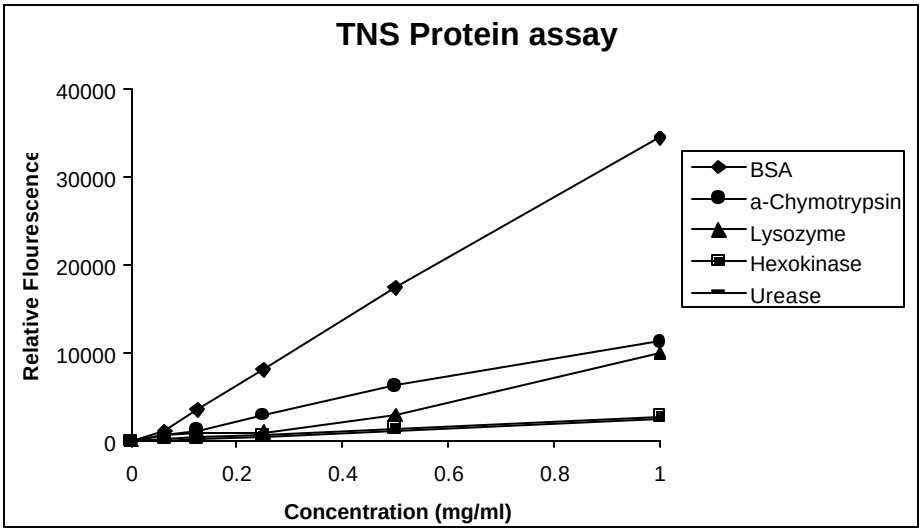

Figure 1. Assay performed with proteins in native confirmations (Buffer A).

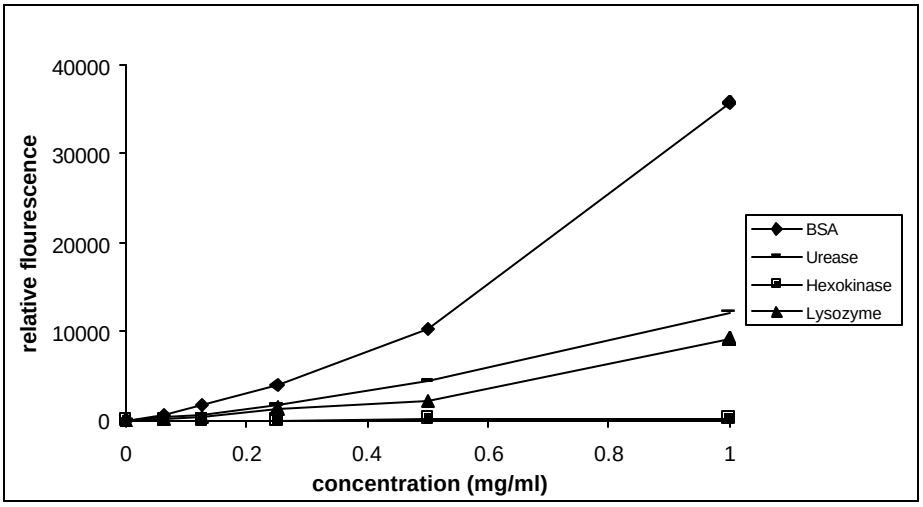

Figure 2. Assay with denaturing reagent SDS (sodium dodecylsulfate) (Buffer B).

IV. SDS-PAGE ANALYSIS

Analysis of protein samples by gel electrophoresis can be performed using the general procedure described as follows, or a modification of this technique. Fluorescent reagent **(B)** (5mM TNS K+/H_2O) should be diluted with water ten fold to 0.5mM. A 12% separating gel containing 3.35mL 0.5mM TNS K+/H_2O, 2.5mL 1.5M TRIS-HCl, pH 8.8, 100µL 10% (w/v) SDS, 4.0mL Acrylamide/Bis (30% stock, degassed for 15 minutes), 50µL 10% ammonium persulfate (APS) (fresh), and 5µL TEMED should be prepared, transferred to the gel sandwich, and immediately overlayed with water. The gel should

be allowed to polymerize for 1 hour, and the overlay solution rinsed off. A 4% stacking gel containing 6.1mL water, 2.5mL 0.5M TRIS-HCl, pH 6.8, 100µL 10% (w/v) SDS, 1.33mL Acrylamide/Bis (30% stock), 50µL 10% APS (fresh), and 10µL TEMED should be poured over an 8-position comb in the gel sandwich until full, and the gel should polymerize for 1.5 hours. A sample buffer containing 3.8mL H_2O, 1.0mL 0.5M TRIS-HCl, pH 6.8, 0.8mL glycerol, 1.6mL 10% (w/v) SDS, 0.4mL 2-mercaptoethanol, 0.4mL 1% (w/v) bromophenol blue should be prepared, and protein samples should be dissolved in Buffer A to yield a concentration of 2mg/mL. 10-15µL of each protein sample diluted 1:1 with sample buffer, or as desired, should be loaded into appropriate lanes of gel. Upper and lower buffer chambers should be filled with a 1X electrode buffer previously diluted from a 5X buffer, pH 8.3, containing TRIS (15g/L), glycine (72g/L), and SDS (5g/L), and gels run at 200V, constant voltage, for 45 minutes or as required. After observing fluorescence, gels can be stained with 0.1% Coomassie blue in a fixing solution of 40% MeOH/10%HOAc/ H_2O for thirty minutes, destained with several changes of fixing solution, rinsed, and stored at 4°C.

REFERENCES

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Kit Contents

Description	Quantity	Part No.	Storage
Sample preparation			
Buffer A: 20mM TRIS, pH 7.5, 150mM NaCl, 5mM 2-mercaptoethanol	20mL	0794-001	C,G
Buffer B: 20mM TRIS, pH 7.5, 150mM NaCl, 5mM 2-mercaptoethanol, 0.1% SDS	20mL	0794-002	C,G
1mM TNS(free acid)/10% DMSO	10mL	0794-003	C,G,L
Standard A: BSA in TRIS Buffer, 1mg/mL	5mL	0794-004	C,G
Standard B: BSA in TRIS Buffer w/ 0.1% SDS, 1mg/mL	5mL	0794-005	C,G
5mM TNS K+/H_2O	1.75mL	0794-006	C,G,L
MSDS sheets	2	0794-007	N/A
Product Information sheet	1	0794-008	N/A

Notes: F=store at or below -18^{o}C; R=store at room temperature; C=store cold (4^{o}C); L=light sensitive; D=store desiccated; FL=flammable; G=wear protective clothing/gloves/safety glasses when using; B=avoid breathing dust/fumes.