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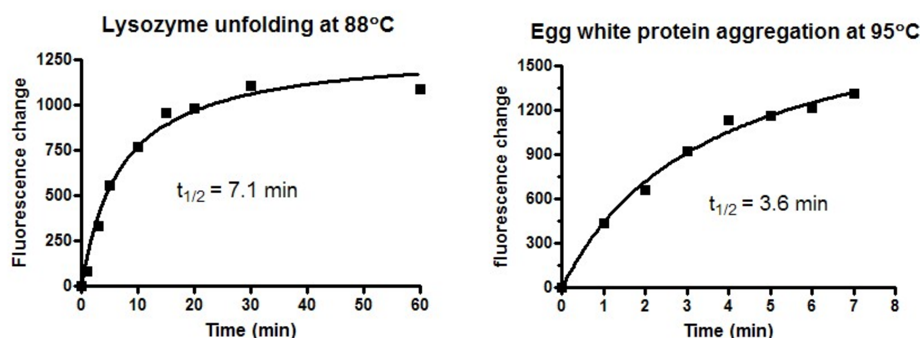
INSTRUCTIONS

ProFoldin
Protein Stability and Aggregation Assay Kit

Catalog Number PSA200K

INTRODUCTION

The Protein Stability and Aggregation Assay Kit is for evaluation of protein thermal stability and analysis of protein unfolding aggregation. The assay is based on a fluorescence dye binding to the hydrophobic surfaces of the proteins and generates fluorescence at 610 nm. Since the unfolded (denatured) proteins have more hydrophobic surfaces than the native ones, the unfolded protein generates a higher fluorescence intensity than the native protein does at the same protein concentration in the same buffer. By incubation of the native protein at a raised temperature, a thermal unfolding curve can be observed and the half life time ($t_{1/2}$) of the protein is calculated. The half life time value can be used to evaluate protein stability such as comparison between the wild-type and mutant proteins. The thermal unfolding curve can also be used to analyze the protein unfolding aggregation state of protein samples.



Each kit (Catalog number PSA200K) contains 60 µl of 1000 x PSA dye for more than 200 assays with the assay volume of 0.3 ml using 96-well plates or cuvettes.

References:

1. Takahashi N et al, TRPV4 channel activity is modulated by direct interaction of the ankyrin domain to PI(4,5)P_2. Nat Commun. 26;5:4994 (2014).
2. Srinivasan S. et al, Hypoxia-induced expression of phosducin-like 3 regulates expression of VEGFR-2 and promotes angiogenesis. Angiogenesis 18:449–462 (2015).
3. Matsuura, S et al; Enzyme Immobilization in Mesoporous Silica for Enhancement of Thermostability. Journal of Nanoscience and Nanotechnology, Volume 18, Number 1, pp. 104-109 (2018).

PROTOCOL - Protein Thermal Unfolding Curve and Protein Stability

The protein sample can be a purified protein or a protein mixture. The protein solution and buffer should not contain any detergent. Most proteins unfold at a temperature between 50 and 100 °C within 15 min.

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Select an appropriate temperature for the unfolding reactions to complete in the time scale of 5 min to 30 min for the convenience of measurement.

1. Prepare 1 ml of 0.5 mg/ml protein solution. Divide the protein solution evenly in 9 aliquots (110 µl each). Keep the samples on ice.
2. Keep one sample on ice as a time zero control. Incubate the other 8 samples in a water-bath at the selected temperature and start to record the incubation time. Move one sample from the water-bath to ice at each time point until all the samples are collected. Transfer 100 µl of each sample into the wells of a black 96-well plate.
3. Mix 3 µl of the 1000 x PSA dye with 3 ml of water to make the 1 x PSA dye. Add 200 µl of the 1 x PSA dye into the wells with the protein samples. Incubate the mixtures at room temperature for 5 min.
4. Read the fluorescence intensity at 610 nm (excitation at 550 nm).

Data Processing

Calculate the half life time $\mathbf{t_{1/2}}$ using the following curve fitting formula:

$$\textbf{Fluorescence change}\ \ \Delta F = F - F_N = [F_U \times t / (t_{1/2} + t)]$$

where **t** is the incubation time of the protein at the selected temperature; **F** is the fluorescence of the sample at time **t**; $\mathbf{F_N}$ is the fluorescence of the unheated sample (**t = 0**); $\mathbf{t_{1/2}}$ is the half life time; $\mathbf{F_U}$ is the maximum fluorescence of the curve representing the fluorescence of the completely unfolded (denatured) protein. The **F,** $\mathbf{F_N}$ and **t** values are from the measurement. The $\mathbf{t_{1/2}}$ and $\mathbf{F_U}$ values are obtained from the fitting. Standard curve fitting programs for binding Kd or enzyme Km calculation can be used to calculate the $\mathbf{t_{1/2}}$ and $\mathbf{F_U}$ values.

Protein Unfolding Aggregation State

An authentic native protein sample is needed to read the background signal. All samples are prepared at the same protein concentration in the same buffer. The buffer should not contain any detergent. The measurement is performed at room temperature.

1. Prepare 100 µl of native protein solution, 100 µl of sample protein solution and 100 µl of unfolded protein solution. The unfolded protein solution is prepared by heating.
2. Dilute 1 µl of the 1000 x PSA dye with 1 ml of water to make the 1 x PSA dye. Mix 200 µl of the 1 x PSA dye with 100 µl of the protein solution for 5 min. Read the fluorescence at 610 nm (excitation 550 nm). Record the fluorescence values:

Sample	Fluorescence
Native protein	$\mathbf{F_N}$
Unfolded protein	$\mathbf{F_U}$
Sample protein	$\mathbf{F_S}$

3. Calculate the percentage of the unfolded protein in the sample protein using the following formula:

$$\text{Unfolded (\%)} = 100\ \% \times (F_S - F_N) / (F_U - F_N)$$

Control Proteins for Unfolding Aggregation Reaction

Commercially available proteins such as lysozyme (Sigma Catalog No. L6876) or lactate dehydrogenase (LDH, Boeringer product) at a concentration of 1 mg/ml can be used as controls. BSA is not a good control.

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