Antibody/Protein Aggregation Assay Kit (BN00567)

(Catalog BN00567; 100 assays; Store at -20°C)

I. Introduction:

Antibody and protein aggregation are important issues in the field of Biotherapeutics. A wide range of conditions and different stages of processing of biopharmaceuticals, such as, formulation, purification, storage, shipping and handling, heat, freeze thaw cycles, mechanical stress, chemical treatment etc. might cause aggregation of proteins, enzymes and antibody targets. The possibility of protein aggregates in eliciting immunogenicity is a major concern for biotherapeutics, which might compromise the safety and efficacy of the biologically derived drug products. Recombinant protein production and storage would highly benefit from a high throughput screening method that can screen a variety of formulation and storage conditions of a particular target protein/antibody. Currently, different biophysical techniques such as Dynamic Light Scattering (DLS), Size-exclusion Chromatography with Multi-angle Light Scattering (SEC-MALS) can detect the presence of small amount of aggregates in a protein/antibody preparation, but they are not amenable for high-throughput screening format. Assay Genie's Antibody/Protein Aggregation assay kit is a platform to estimate the extent of aggregation within a protein/antibody sample. This is a fast, high-throughput, plate-based fluorometric assay format and can detect as low as 0.5% antibody aggregate.

Non-Aggregated Antibody —(Aggregation Assay Probe)→ No / Very low Fluorescence (Ex/Em: 440/500)

Aggregated antibody —(Aggregation Assay Probe)→ Fluorescence (Ex/Em: 440/500)

II. Applications:

- Detection of aggregates in antibody and protein samples.
- Detection of protein aggregation during formulation and long terms storage of recombinant proteins and enzymes.
- Screening of buffer conditions for optimum refolding of a recombinant protein/enzyme.

III. Sample Type:

- Antibody/Protein/Enzyme solutions

IV. Kit Contents:

Components	BN00567	Cap Code
Aggregation Assay Buffer	12 ml	WM
Antibody aggregate standard (0%)	1500 µl	White
Antibody aggregate standard (10%)	1500 µl	Orange
Aggregation Assay Probe	20 µl	Red

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrofluorometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Aggregation Assay Buffer:** Ready to use. Warm to room temperature before use. Store at 4ºC.
- **Antibody aggregate standards**: Store at -20°C.Thaw before use. Use within two months. Antibody Aggregate Standards should be aliquoted and stored at -20°C; avoid multiple freeze thaws.
- **Aggregation assay probe:** Thaw before use. Add 198 µl of anhydrous DMSO to the vial, mix by pipetting. Store probe at -20°C. Use within two months.

VII. Antibody aggregation Assay Protocol:

1. **Sample Preparation:** Pipette 100 µl each of Aggregation Assay Buffer (**Blank, BK**), Buffer in which antibody/protein is prepared (**Buffer Control**, **BC**) and test antibody solution (**Sample, S**) in different wells of a 96-well white plate. Add 2 µl of the diluted Aggregation Assay Probe to each of the well(s). Incubate for 15 min at room temperature in the dark.

   Notes:

   For proper quantification of aggregation, perform a parallel **Sample Control (SC)** with a freshly made non-aggregated sample of same concentration

2. **Antibody Aggregation Standard Curve:** Add 0, 20, 40, 60, 80, and 100 µl of Antibody Aggregation Standard (10%) respectively to a 96-well plate to generate 0, 2, 4, 6, 8 and 10% Aggregation Standards. Adjust the volume to 100 µl with Antibody Aggregation Standard (0%) and mix them properly.

   Notes:

   a. Assay Genie's antibody aggregate standards are provided at 5 mg/ml (final amount: 500 µg antibody/well). They also have been tested with stock concentration of as low as 1 mg/ml (final 100 µg antibody/well).

   b. For quantitative measurement of aggregation, it is recommended to use antibody sample solutions of 5 mg/ml or higher and dilute the sample with Aggregation Assay Buffer to reach a final antibody concentration of ~5 mg/ml.
   For best results with a particular antibody/protein target, we recommend preparing your own aggregation standard curve by mixing 100% aggregated target with 100% non-aggregated protein in appropriate amounts.

3. **Antibody Aggregation Probe:** Add 2 µl of the Aggregation assay probe to each of the well(s) containing the standards, samples, buffer control, blank, and Sample Controls. Mix well. Incubate for 15 mins at room temperature in dark.
4. **Measurement:** Measure Fluorescence at (Ex/Em 440/500 nm) in end point mode at RT.
5. **Calculation:** Subtract 0 Standard reading (RFU) and **SC** reading from all **Standard** and **Sample** readings respectively. Plot the Antibody Aggregation Standard Curve by plotting ΔRFU vs % of aggregate standard. If **BC** reading is significant, subtract the **BC** reading from its paired sample reading. Calculate % aggregation in the antibody sample using the standard curve. $\Delta RFU(y) = RFU_{Sample} - RFU_{Sample\ Control}$. Apply the ΔRFU to the Antibody Aggregation Standard Curve to get percentage of aggregation.

$$\%\ \boldsymbol{Aggregation\ in\ the\ sample}\text{: } \boldsymbol{X} = \frac{(y)}{a}\ \%$$

Where: **y** = ΔRFU of sample
X = Percentage Aggregation
a = Slope of the standard curve

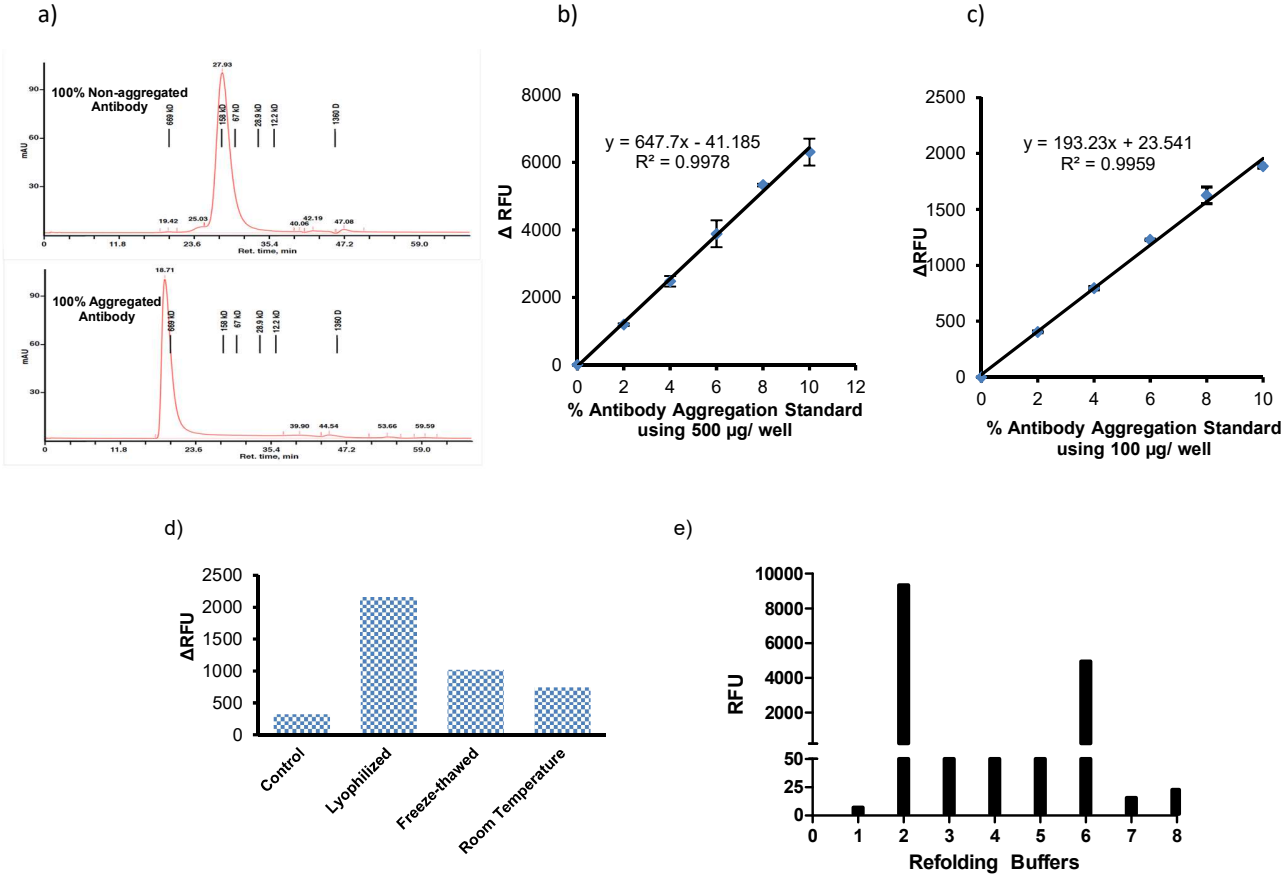

Figure: (a) Size Exclusion Chromatography of 100% non-aggregated antibody and 100% aggregated antibody samples are premixed in appropriate ratio and provided as 0-10% Antibody Aggregate Standards (b) % Aggregation Standard Plot using Assay Genie's Antibody Aggregate Standards (with 5 mg/ml, final amount: 500 µg/well (c) % Aggregation Standard Plot obtained using Assay Genie's Antibody Aggregate Standards (1 mg/ml, final amount: 100 µg/well) (d) Protein Aggregation detected after a proprietary protein sample (10 mg/ml) was subjected to different formulation and storage conditions, (e) Protein Aggregation detected after refolding of denatured lysozyme in using different buffer formulations. The assays were performed as indicated in the protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.