Version 1 Last updated 25 April 2018

ab234048 Protein Aggregation Assay Kit

For the measurement of antibody/protein aggregation within a antibody/protein/enzyme solution.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Protein Aggregation Assay Kit (ab234048) is a platform to estimate the extent of aggregation within a protein/antibody sample.

This is a fast, high-throughput, plate-based fluorometric assay that can detect as low as 0.5% antibody aggregate.

Prepare all standards and samples as directed.

Pipette 100 µL of each standard and sample into different wells of a 96-well plate.

Add aggregation probe to each well and incubate for 15 minutes at room temperature in the dark.

Measure the fluorescence at Ex/Em 440/500 nm.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Aggregation Assay Buffer	12 mL	-20°C	4°C
Antibody aggregate standard (0%)	1500 µL	-20°C	-20°C
Antibody aggregate standard (10%)	1500 µL	-20°C	-20°C
Aggregation Assay Probe	20 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrofluorometer capable of measuring fluorescence at Ex/Em = 440/500 nm
- 96-well white plate with clear flat bottom.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Aggregation Assay Buffer

1. Ready to use as supplied. Warm to room temperature before use.

5.2 Antibody aggregate standard (0%)

1. Ready to use as supplied. Thaw before use.

5.3 Antibody aggregate standard (10%)

1. Ready to use as supplied. Thaw before use.

5.4 Aggregation Assay probe

1. Thaw before use.
2. Add 198 µL of anhydrous DMSO to the vial, mix by pipetting.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

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.

Standard #	Antibody Aggregation Standard 10% (µL)	Antibody Aggregation standard 0% (µL)	Final volume standard in well (µL)	End amount Antibody Aggregation Standard in well (%)
1	0	100	100	0
2	20	80	100	2
3	40	60	100	4
4	60	40	100	6
5	80	20	100	8
6	100	0	100	10

ΔNote: The 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).

ΔNote: 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.

ΔNote: 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.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

- Pipette 100 µL each of Aggregation Assay Buffer, Buffer in which antibody/protein is prepared (Buffer control) and test antibody solution 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 minutes at room temperature in the dark.

ΔNote: For proper quantification of aggregation, perform a parallel Sample Control with a freshly made non-aggregated sample of same concentration.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Antibody Aggregation probe:

1. Add 2 µL of the Aggregation assay probe to each of the wells containing the standards, samples, buffer control, blank and sample controls.
2. Mix well and incubate for 15 minutes at room temperature in the dark.

8.2 Measurement:

1. Measure fluorescence at Ex/Em 440/500 nm in end point mode at room temperature.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank and sample control from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the Antibody Aggregation Standard Curve by plotting ΔRFU vs % of aggregate standard.
5. If the Buffer control reading is significant, subtract the Buffer Control reading from its paired sample reading.
6. Calculate % aggregated in the antibody sample using the standard curve, $\Delta RFU(y) = RFU_{Sample} - RFU_{Sample\ Contol}$
7. Apply the ΔRFU to the Antibody Aggregation Standard Curve to get percentage of aggregation.

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

Where: **y** = ΔRFU of sample

X = Percentage of Aggregation

a = Slope of the standard curve

10. Typical Data

Data provided **for demonstration purposes only.**

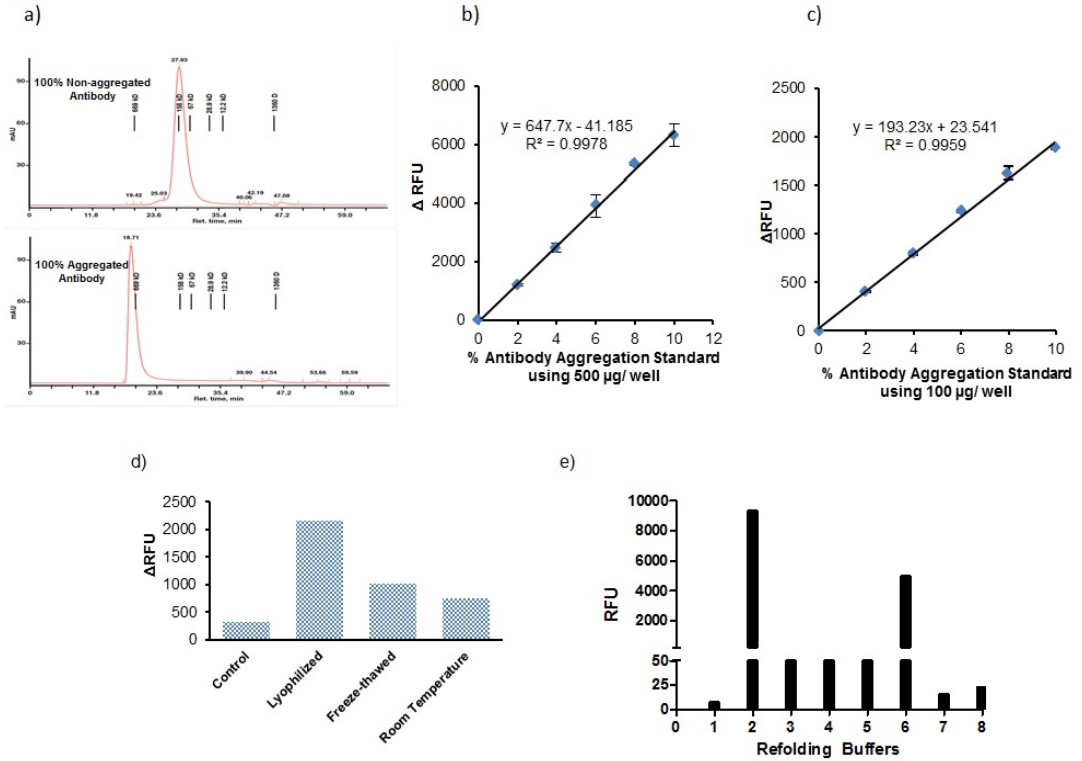

Figure 1: (a) Size Exclusion Chromatography of 100% non-aggregated antibody and 100% aggregated antibody samples are premixed in appropriate ratio and provided as 0-10% Antibody Aggregate Standards. **(b)** % Aggregation Standard Plot using the Antibody Aggregate Standards (with 5 mg/mL, final amount: 500 µg/well. **(c)** % Aggregation Standard Plot obtained using the 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 lysozymes using different buffer formulations. The assays were performed as indicated in the protocol.

11. Notes

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