QRET Technologies Ltd.

Protein-Probe Aggregation Assay kit

INTRODUCTION

- A highly sensitive mix-and-measure assay
- Performs with a wide scale of excipients
- High throughput 384-microplate format

Protein-Probe aggregation assay provides a user-friendly approach to monitor protein aggregation in solution. This is useful for defining optimal storage formulations to proteins. Protein-Probe is highly sensitive and 50-fold more sensitive than thermofluor assays using SYPRO Orange. Subsequently, the Protein-Probe assay is far more sensitive than methods such as UV detection, dynamic light scattering (DLS) and size-exclusion chromatography. Protein aggregation is also detected nearly 10-fold earlier than with the reference methods. Therefore, Protein-Probe in combination with the high throughput detection format provides high sensitivity approach with lowered protein consumption and early detection of aggregation.

PRODUCT

Protein-Probe Aggregation Assay kit

The given condition is optimized for assaying antibody aggregation.

PRODUCT MATERIALS

Kit provides 400 assay points in a 384 well plate format.

1. Buffer solution
2. Modulation solution
3. Eu-probe solution

Stored at +4 °C

Additionally, deionized water is required.

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INSTRUMENT

Plate reader equipped with time-resolved luminescence (fluorescence) detection mode is required.

PROTOCOL

The solution preparation for 100 assay wells

Detection solution

1. Dilute the concentrated Buffer solution 650 μL by adding 5700 μL deionized water
2. Add 75 μL of the Modulation solution and mixed the solution
3. Add 75 μL of the Eu-probe solution
4. Mix the prepared **Detection solution**

Assaying

5. Dispense 65 μL of the Detection solution to a 384 microtiter well
   (ensure that the plate well allows a total volume of at least 80 μL)
6. Add 2 μL of sample solution to the Detection solution
   (sample solution concentration approximately 5 mg/mL)

Signal reading

7. Shake gently for 10 s in the microtiter plate shaker
8. Measure for time-gated europium signal with plate reader using
   - Delay time 800 μs
   - Window time 400 μs
   - Excitation wavelength 340 nm
   - Emission wavelength 620 nm

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EXEMPLARY ASSAY

Compare the europium luminescence signal of the sample to a known non-aggregated sample. Higher luminescence signal of the sample indicates protein aggregation. Typically, non-aggregated antibody solution gives low signal and equal to the signal of the Detection solution. In the Figure below aggregated Trastuzumab was added to a known non-aggregated Trastuzumab solution. The obtained luminescence signal is background subtracted with the luminescence signal of the non-aggregated solution.

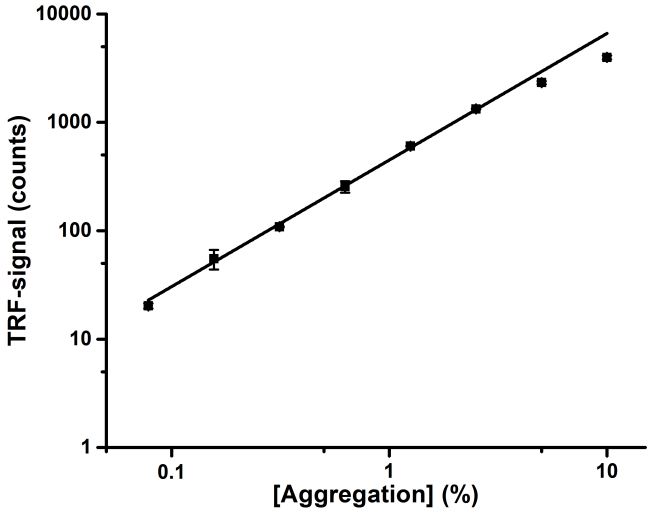

Figure. Trastuzumab aggregation measurement. Aggregated Trastuzumab was mixed with non-aggregated Trastuzumab keeping the total concentration at 5 mg/mL. X-axis represent %-aggregate within non-aggregated sample and y-axis is background subtracted time-gated europium luminescence signal.

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