Mar 27, 2025

# Amyloid beta (M1-42) Aggregation Monitored by Thioflavin-T (ThT) Fluorescence in a Plate Reader

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l68ky5gqe/v1

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**Protocol Citation:** Patricia Yuste-Checa, F Ulrich Hartl 2025. Amyloid beta (M1-42) Aggregation Monitored by Thioflavin-T (ThT) Fluorescence in a Plate Reader. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l68ky5gqe/v1



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** March 20, 2025

**Last Modified:** March 27, 2025

**Protocol Integer ID:** 124796

**Keywords:** ASAPCRN, amyloid beta, fluorescence, plate reader, thioflavin, aβ

**Funders Acknowledgements:**
**Aligning Science Across Parkinson's**
Grant ID: ASAP-000282

## Abstract

This protocol is based on Linse S. *Methods Mol Biol*. (2020), section "3.10 Preparation of Samples for Kinetics" and details how to efficiently monitor Aβ(M1–42) aggregation by thioflavin T fluorescence in a plate reader.

## Materials

**Buffers**:

- **Gu6**: pH pH 8.5

| | A | B |
|---|---|---|
| | Guanidinium hydrochloride | 6 M |
| | Sodium phosphate | 20 mM |

- **PE 8.5**: pH pH 8.5

| | A | B |
|---|---|---|
| | Sodium phosphate | 20 mM |
| | EDTA | 0.2 mM |

- [M] 1 millimolar (mM) Thioflavin T (ThT) in water. This can be stored at -20 °C .

**Equipment**

- CLARIOstar plate reader (BMG Labtech)

Corning® 96-well Half Area Black/Clear Flat Bottom Polystyrene NBS Microplate, 25 per Bag, without L **Corning Catalog #**3881

## Size Exclusion Chromatography

1

Note

To discard any possible aggregate that could have formed during the freezing lyophilization process, size exclusion chromatography of the purified peptide should be performed before the kinetic experiment.

Dissolve 0.5 mg of lyophilized Aβ (M1–42) in 700 µL Gu6 buffer.

Note

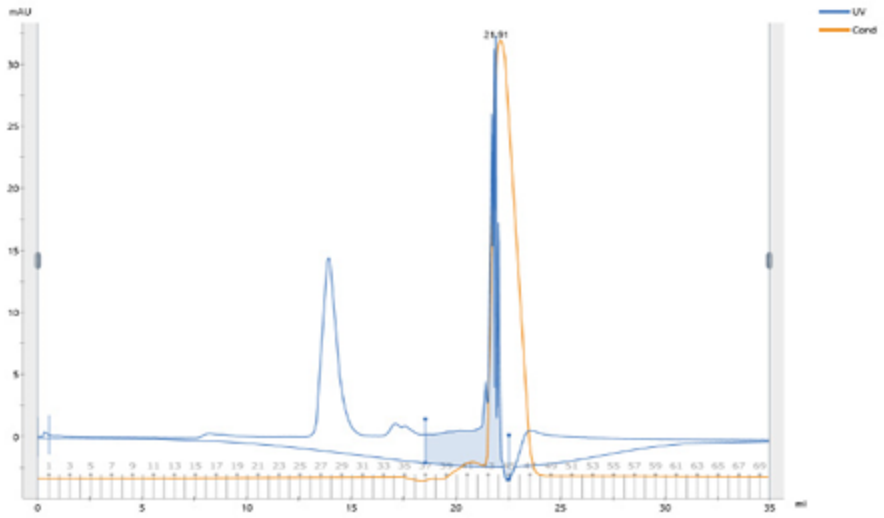
See protocol “Purification of recombinant Amyloid beta peptide (M1-42) from *Escherichia coli*”.

2 Load the sample onto a Cytiva Superdex 75 Increase 10/300 GL ( 24 mL ) SEC column previously equilibrated with PE 8.5 buffer.

3 Run the column with PE 8.5 at 0.5 µL .

Note

**NOTE**: Collect the fractions preferably in low binding tubes.




**Size exclusion chromatogram**

4 Aβ(M1–42) monomers elute at around 14-15 mL (fractions around 27-31).

5 Measure absorbance of fractions at 205 nm wavelength using a UV spectrophotometer using PE 8.5 buffer as blank.

Note

**NOTE**: Aβ (M1-42) contains just one tyrosine and no tryptophan. Therefore, measurement at 280 nm wavelength of low concentrated peptide may not be accurate.

6 Pool fractions containing Aβ(M1–42) monomers and measure the concentration at $A_{205nm}$.

7 Prepare aggregation reactions:

- Mix reagents to a final concentration of [M] 4 micromolar (µM) Aβ(M1–42), [M] 10 micromolar (µM) ThT in PE 8.5 buffer.
- Prepare a mix for 4.5 reactions per condition (quadruplicates in each plate as technical replicates), final volume 80 µL per well.

Note

Mix of 360 µL = 80 µL reaction x 4.5.

- Concentration of Aβ(M1−42) and ThT should be tested empirically in order to achieve a final concentration in a range that provides a linear response of the fluorescence intensity versus fibril mass concentration, see Linse S. Methods Mol Biol. (2020).
- Molecular or chemical chaperones can be included in the reaction in order to study their effect on Aβ(M1−42) aggregation.
- Keep Aβ(M1−42) peptide as well as the 96 well plate during preparation always on ice.

8 Dispense 80 µL of the mix into a well of a 96-well half-area plate of black polystyrene with a clear bottom (Corning 3881).

9 If possible, the outer wells should not be used and should be filled with water.

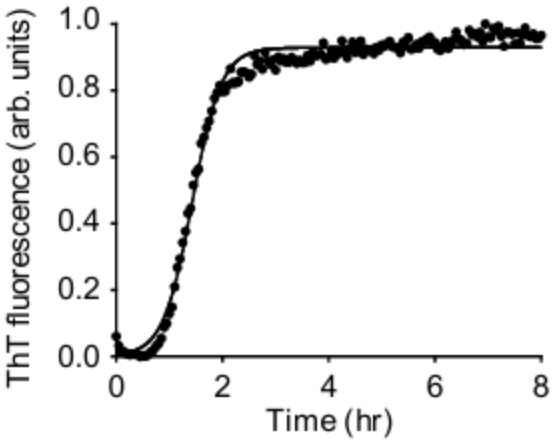
10 Seal the plate with parafilm to avoid excessive evaporation.

11 In a CLARIOstar plate reader (BMG Labtech) set the following parameters and start the reaction:

- Fluorescence measurement: ThT signal, excitation 440 nm, emission 480 nm, measured every 00:03:00 .
- Temperature 37 °C .

Note

**NOTE**: Under these conditions Aβ(M1−42) rapidly aggregates, reaching a fluorescence plateau after approximately 02:00:00 aggregation. The data can be fitted using Sigma plot software (Sigmoidal, Sigmoid, 3 Parameter function) to obtain the half time for reaching the aggregation plateau.




## Protocol references

Linse S. Expression and Purification of Intrinsically Disordered Aβ Peptide and Setup of Reproducible Aggregation Kinetics Experiment. Methods Mol Biol. 2020;2141:731 754. doi: 10.1007/978-1-0716-0524-0_38. PMID: 32696387.