



SensoLyte® Thioflavin T [Pyr3] - β -Amyloid (3-42) Aggregation Kit **Fluorimetric**

Revision #: 1.0

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Catalog #	AS-72276
Kit Size	96-well plate format

- **Optimized Performance:** Optimal conditions for [Pyr3] - β -Amyloid (3-42) fibrillation.
- **High Speed:** Minimal hands-on time.
- **Assured Reliability:** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Assay Buffer	25 ml
Component B	AggreSure™ [Pyr3]-A β (3-42)	0.5 mg (2 x 0.25 mg, net peptide)
Component C	Thioflavin T (ThT)	20 mM, 100 μ l
Component D	Morin	20 mM, 25 μ l
Component E	Phenol Red	20 mM, 25 μ l
Component F	Solvent Buffer	1%, 300 μ l

Other Materials Required (but not provided)

- 96-well plate: non-binding black microplate.
- Fluorescence microplate reader: Capable of detecting emission at 484 nm with excitation at 440 nm.
- The amount of AggreSure™ [Pyr3]-A β (3-42) supplied with the kit is enough for 20 assays and can be purchased separately (Cat. AS-72277). All other components are supplied to run 100 assays.

Storage and Handling

- Store kit components at 4 °C.
- Components B, D, and E should be stored at -20 °C if not used within one week.

Introduction

Pyroglutamate modification of β -amyloid is one of the predominant amyloid peptide structures deposited in human brain of Alzheimer's disease and Down's syndrome patients¹. The Pyro-Glu modification on the N-terminal of β -amyloid at position 3 provides resistance to degradation². It also has higher toxicity and aggregation propensity compared to non-modified beta-amyloid. Many studies suggest that this peptide can form toxic oligomers and fibrils under physiological conditions and rapidly aggregate³. Since [Pyr3]-A β (3-42) aggregation is evidently an essential event in the pathogenesis of AD, a reliable assay is important to study A β fibrillation kinetics and screen for A β aggregation inhibitors.

SensoLyte[®] ThT [Pyr3]-A β (3-42) Aggregation kit provides a convenient and standard method to measure [Pyr3]-A β (3-42) aggregation using Thioflavin T dye. The peptide, provided in the kit, is pretreated to ensure it is in a monomer state. Optimized fibrillation buffer is included with the kit, and two known inhibitors are supplied as a control. The assay is based on the property of ThT dye to increase its fluorescence Ex/Em=440/484 nm when bound to aggregated A β peptides.

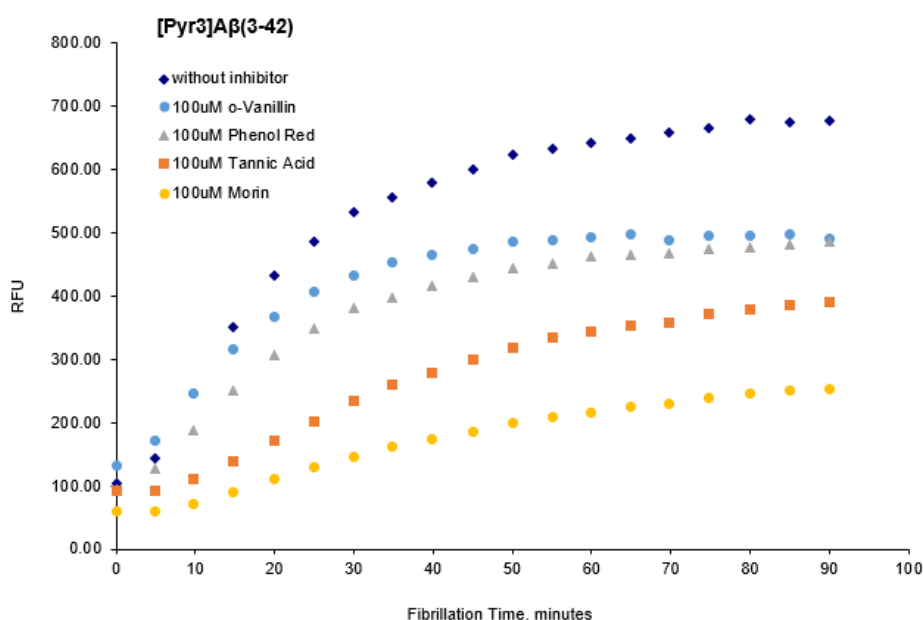


Figure 1. An increase of fluorescence signal is correlated with an increase of [Pyr3]-A β (3-42) fibril formation. Phenol Red, Morin, Tannic Acid and o-Vanillin were added at 100 μ M final concentration to inhibit [Pyr3]-A β (3-42) aggregation. Fluorescence signal was monitored at Ex/Em= 440/484 nm every 5 minutes at 37 °C with 15 seconds shaking between reads (SpectraMax M5e, Molecular Devices).

Protocol

Note 1: The assay were only tested with assay buffer 50mM Tris/150mM NaCl (pH=7.2) and may not fibrillate in other buffer systems

Note 2: The amount of [Pyr3]-A β (3-42) supplied with the kit is enough for 20 assays and can be purchased separately (Cat. AS-72277). All other components are supplied to run 100 assays.

Use only non-binding black plates to run assay, plates that absorb proteins will affect assay performance!

1. Prepare working solutions

Note: Warm up all kit components, except Component A, to room temperature.

Component A should be kept at 4 °C to prevent premature [Pyr3]-A β (3-42) aggregation.

1.1 ThT working solution: Add 100 μ l of 20mM ThT (Component C) to 900 μ l of the assay buffer (Component A) to get 2mM solution. This amount is enough for 100 assays. Adjust amount of 2mM ThT for the number of assays if necessary.

1.2 [Pyr3]-A β (3-42) peptide solution: Add 100 μ l **cold** solvent buffer (Component F), then add 900 μ l of **cold** assay buffer (Component A) to AggreSure[™] [Pyr3]-A β (3-42) glass vial

(Component B). Let peptide to hydrate for a few minutes. Sonicate (water bath sonicator) for 3-5 minutes if necessary to completely dissolve peptide. Do not vortex, mix by inversion only. If concerning about peptide solubility, transfer [Pyr3]-A β (3-42) peptide solution into centrifuge tube and spin at 10,000 rpm for 5 minutes at 4 °C to centrifuge out any precipitated material.

- 1.3 **Inhibitor solutions:** Dilute inhibitors (Components D and E) from 20mM stock solution to 2mM in the assay buffer (Component A). 100 μ l of the assay mixture will require 5 μ l of the diluted inhibitor to achieve 100 μ M final concentration. Add 5 μ l of the 2mM inhibitor solution into each of the inhibitor control well.

2. Set up the fibrillation reaction

- 2.1 Add 10 μ l of 2mM ThT (from Step 1.1) into each well.
- 2.2 Add test samples and controls to the microplate wells. Suggested volume of [Pyr3]-A β (3-42) peptide solution is 85 μ l and 5 μ l of the test compound.
- 2.3 Simultaneously establish the following control wells as necessary:
- Inhibitor control contains [Pyr3]-A β (3-42) peptide (from Step 1.2) and aggregation inhibitor supplied in the kit either Morin or Phenol Red (Components D or E) at 100 μ M final concentration.
 - Positive control contains [Pyr3]-A β (3-42) peptide (from Step 1.2) without inhibitor.
 - Vehicle control contains assay buffer (Component A) and vehicle used in delivering test compound (*i.e.* DMSO, concentration not to exceed 1%).
 - Test Compound contains [Pyr3]-A β (3-42) peptide (from Step 1.2) and test compound.
 - Test Compound Control contains assay buffer (Component A) and test compound only.
 - Blank contains assay buffer (Component A) only.
- 2.4 Bring the total volume of all samples to 100 μ l using assay buffer (Component A).

3. Run Fibrillation Assay

- 3.1 Immediately start measuring fluorescence intensity at 37°C with Ex/Em=440 nm/484 nm and 15 sec shaking between reads to facilitate aggregation. Read every 5 or 10 minutes.
- Note: If temperature control is unavailable in fluorescence reader, incubate plate in 37 °C incubator between reads. Protect plate from light.

4. Analysis of Data.

- 4.1 The fluorescence reading from the blank control wells is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells. All fluorescence readings are expressed in relative fluorescence units (RFU).
- 4.2 Plot fibrillation kinetics curve as RFU versus fibrillation time.
- 4.3 Determine inhibition %, IC₅₀, etc.

References

1. Wirths, O., Bethge, T., Marcello, A., Harmeier, A., Jawhar, S., Lucassen, P. J., Multhaup, G., Brody, D. L., Esparza, T., Ingelsson, M., Kalimo, H., Lannfelt, L., & Bayer, T. A. (2010).
2. D'Arrigo, C., Tabaton, M., & Perico, A. (2009).
3. Piccini, A., Russo, C., Gliozzi, A., Relini, A., Vitali, A., Borghi, R., Giliberto, L., Armirotti, A., D'Arrigo, C., Bachi, A., Cattaneo, A., Canale, C., Torrassa, S., Saido, T. C., Markesbery, W., Gambetti, P., & Tabaton, M. (2005). *The Journal of biological chemistry*, 280(40), 34186–34192.