

Fibrillation assay

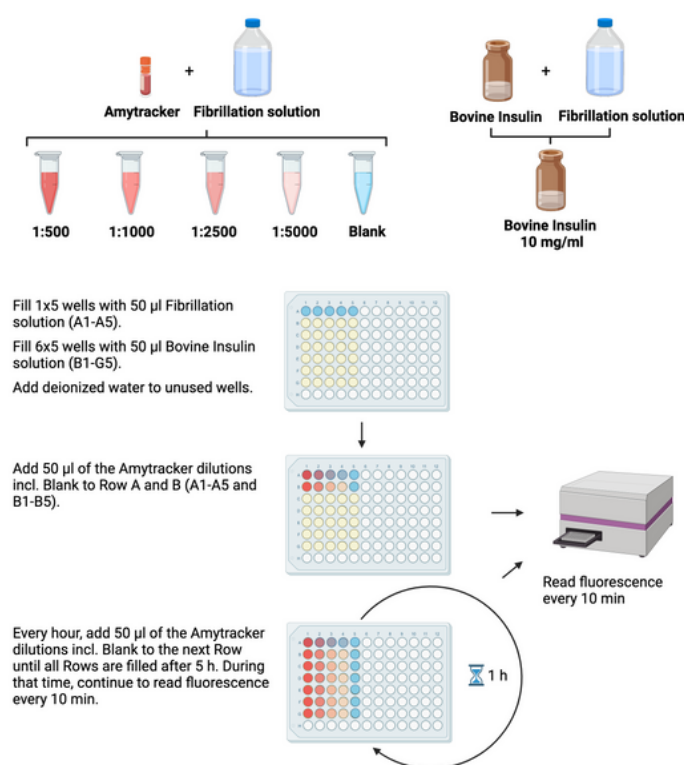
This protocol describes how Amytracker can be utilized for fibrillation assays and detection of amyloids in liquid samples. As all Amytracker variants are highly fluorescent only when they are bound to their target, they are ideally suited for spectrophotometric analysis. We recommend to perform a titration to use Amytracker in the lowest concentration possible for your specific application. The experimental conditions used to induce protein misfolding and aggregation can vary considerably depending on the amyloidogenic protein or peptide. It is important to note that Amytracker fluorescence can vary depending on pH and ionic strength of the buffer. In this protocol, fibrillation of bovine insulin is performed in 2 M acetic acid and 0.5 M NaCl.

Solutions and Reagents:

- **Amytracker - DMSO**
- Fibrillation solution: 2 M acetic acid and 0.5 M NaCl in deionized water
- Bovine insulin
- Deionized water
- 96-well microtiter plate with lid.
- Plate reader

Assay Procedure:

- Prepare a dilution series of Amytracker (1:500, 1:1000, 1:2500, 1:5000) in Fibrillation solution and include a blank control (Fibrillation solution without Amytracker).
- Prepare 10 mg/ml bovine insulin in Fibrillation solution.
- Fill 1x5wells with 50 μ l Fibrillation solution.
- Fill 6x5wells with 50 μ l Bovine Insuline solution.
- Fill the rest of the wells with deionized water to avoid evaporation.
- Add 50 μ l of each Amytracker dilution incl. Blank to Row 1 & 2.
- Place the lid on the microtiter plate and read emission every 10 min for 5 hours in total.
- Every hour, remove the plate from the Reader and add Amytracker dilutions incl. Blank to the next row filled with Bovine Insulin solution.



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Readout:

- Make sure to read from the bottom of the plate. Note that excitation and emission are different for each Amytracker variant and excitation- and emission maximum can slightly vary for each target. For reference excitation and emission settings, see table below.

Optotracing using Amytracker

Amytracker are optotracers with structure-dependent photophysical properties. All Amytracker variants are designed to bind to the Congo red binding pocket on the amyloid fibril and require a theoretical minimum of eight in-register parallel- β -strands for binding. Amytracker reliably binds amyloids derived from a variety of amyloidogenic proteins or peptides from different species. The Amytracker variants are only fluorescent when binding to a target and the specific excitation and emission maxima can vary depending on the target. To investigate different targets, we recommend to perform imaging by exciting the sample with different wavelengths collecting fluorescence intensity in multiple emission ranges (see the table below for reference). Use the QR code to access excitation- and emission spectra for all Amytracker variants.



Scan QR code to see
Amytracker spectra

Table: Amytracker spectral properties with maximum excitation (Ex_{max}) and emission (Em_{max}) when bound and recommended range for acquisition of excitation- and emission spectra as well as recommended filter sets for microscopy.

Amytracker variant	Ex_{max}	Em_{max}	Excitation spectrum (detect at Em_{max})	Emission spectrum (excite at Ex_{max})	Recommended filter sets
Amytracker 480	420 nm	480 nm	300 - 450 nm	450 - 800 nm	DAPI
Amytracker 520	460 nm	520 nm	300 - 490 nm	490 - 800 nm	FITC, GFP
Amytracker 540	480 nm	540 nm	300 - 510 nm	510 - 800 nm	FITC, GFP, YFP
Amytracker 630	520 nm	630 nm	300 - 600 nm	550 - 800 nm	PI, Cy3, TxRed, mCherry, Cy3.5
Amytracker 680	530 nm	680 nm	300 - 650 nm	660 - 800 nm	PI, mCherry, Cy3.5

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