

## Protocol IV: Fibrillation assay

This protocol describes how Amytracker™ can be utilized for fibrillation assays and detection of amyloids in liquid samples. As Amytracker™ molecules 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 insuline is performed in 2 M acetic acid and 0.5 M NaCl.

### Solutions and Reagents:

Use Amytracker™ *ex vivo* variant, which is provided as concentrated solution. The following common reagents are required (not supplied):

- Fibrillation solution: 2 M acetic acid and 0.5 M NaCl in deionized water
- Bovine insulin
- 96-well microtiter plate (round bottom)
- Plate reader

### Assay Procedure:

- Prepare a dilution series of Amytracker™ (1:500, 1:1000, 1:5000, 1:10000, 1:50000) in Fibrillation solution and include a blank control.
- Freshly prepare 10 mg/ml bovine insulin in Fibrillation solution.
- Fill the wells of the microtiter plate with 50 µl Amytracker™ of each dilution as well as blank control.
- Add 50 µl bovin insuline (10 mg/ml in Fibrillation solution) to each well.
- Place microtiter plate into Plate reader and record emission every 10 min for 5 h. Note that excitation and emission are different for each Amytracker™ (see section 'Spectrophotometer Settings').

### Spectrophotometer Settings:

- **Amytracker™ 480:** Excite at 430 nm and collect emission at 480 nm. Optional: Record an emission spectrum (450 - 700 nm) with 430 nm excitation.
- **Amytracker™ 520:** Excite at 470 nm and collect emission at 530 nm. Optional: Record an emission spectrum (490 - 700 nm) with 470 nm excitation.
- **Amytracker™ 630:** Excite at 510 nm and collect emission at 635 nm. Optional: Record an emission spectrum (530 - 800 nm) with 510 nm excitation.
- **Amytracker™ 680:** Excite at 540 nm and collect emission at 680 nm. Optional: Record an emission spectrum (560 - 800 nm) with 540 nm excitation.

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#### Head Office

Ebba Biotech AB  
Medeon Science Park  
Per Albin Hanssons väg 41  
SE-205 12 Malmö  
Sweden

#### Stockholm Branch

Ebba Biotech AB  
Karolinska Institutet Science Park  
Fogdevreten 2a  
SE-171 65 Solna  
Sweden

#### Company Information

Org-nr: 559016-7093  
VAT-nr: SE 559016709301

#### Contact

Web: [ebbabiotech.com](http://ebbabiotech.com)  
Email: [info@ebbabiotech.com](mailto:info@ebbabiotech.com)  
Phone: +46 73 985 40 51