

Protocol I: Staining of amyloids in tissue sections

Amytracker™ can be used to stain tissue sections prepared by the most common techniques like paraffin embedding and freezing. Formalin fixation works well for extracellular deposits, and fixation in ice-cold ethanol or acetone is recommended for best preservation of intracellular aggregates. Amytracker™ can be easily combined with your co-staining of choice as described in Protocol II: Antibody co-staining. As Amytracker™ are highly fluorescent only when bound to their target, washing steps might be omitted when working with sensitive tissues. It is not necessary to guard Amytracker™ from light, so incubations can be performed on the bench at room temperature. It is however crucial not to let Amytracker™ dry on the sample.

Solutions and Reagents:

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

- ice-cold Ethanol, 95%
- Phosphate buffered saline (PBS), pH 7.4
- Deionized water
- Mounting medium

Assay Procedure:

- Fix tissue sections with method of choice. We recommend fixation with ice-cold ethanol (5 min) at room temperature.
- Rehydrate tissue sections in a mix of ethanol and deionized water (1:1) for 5 min. The rehydration step may need to be repeated with lower ethanol ratio depending on the tissue.
- Equilibrate sections in PBS for 5 min.
- Dilute Amytracker™ in PBS 1:1000.
- Apply diluted Amytracker™ generously. Use enough liquid to prevent the sections from drying out during incubation. Incubate for 30 min.
- Wash 2 x 5 min in PBS.
- Mount tissue sections and seal the coverslip onto the slide to prevent drying.

Fluorescence Microscopy:

- **Amytracker™ 480:** Excite at 405 nm (standard laser line) and detect emission using the DAPI or FITC filter sets. Optional: An excitation range of 405-458 nm and a detection range of 470–550 nm may be applied depending on available laser lines and filter sets.
- **Amytracker™ 520:** Excite at 458 or 488 nm (standard laser lines) and detect emission using a standard FITC filter set. Optional: An excitation range of 405-488 nm and a detection range of 500-600 nm may be applied depending on available laser lines and filter sets.
- **Amytracker™ 630:** Excite at 488 or 514 nm (standard laser lines) and detect emission using standard TRITC or TxRed filter set. Optional: An excitation range of 458-514 nm and a detection range of 600-650 nm may be applied depending on available laser lines and filter sets.
- **Amytracker™ 680:** Excite at 561 nm (standard laser line) and detect emission using a standard Cy5 filter set. Optional: An excitation range of 530-565 nm and a detection range of 600-800 nm may be applied depending on available laser lines and filter sets.

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