

Protocol II: Staining of amyloids with Amytracker™ and antibody co-stain

Amytracker™ might be combined with antibody staining to confirm presence of specific amyloidogenic proteins or peptides. This protocol describes a simple procedure of how to combine Amytracker™ with antibody staining using a sequence specific primary antibody and a fluorescently labeled secondary antibody. We assume you are using formalin-fixed, paraffin embedded tissues and that tissue sections were deparaffinized and rehydrated prior to staining. Please make sure to select a secondary antibody with suitable fluorescent label (see below in section 'Fluorescence Microscopy'). We recommend a simple blocking/permeabilization procedure. Consider to adjust the blocking and permeabilization steps if necessary.

Solutions and Reagents:

Use Amytracker™ *ex vivo* variant, which is provided as 1000-fold concentrated solution. The following common reagents are required (not supplied):

- Phosphate buffered saline (PBS), pH 7.4
- Blocking/Permeabilization buffer, 4% BSA in PBS with 0.2% Triton-X
- Primary antibody
- Secondary antibody with fluorescent label
- Mounting medium

Assay Procedure:

- Block tissue sections in Blocking/Permeabilization buffer for 30 min at room temperature.
- Incubate tissue sections with primary antibody diluted in Blocking/Permeabilization buffer at 4 °C over-night.
- Rinse with Blocking/Permeabilization buffer.
- Incubate tissue sections with secondary antibody diluted in Blocking/Permeabilization buffer at room temperature for 30 min.
- Rinse with Blocking/Permeabilization buffer.
- Wash 5 min in PBS.
- 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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