Protocol I: Staining of protein aggregates 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 protect **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 (optional).
- Mount tissue sections and seal the coverslip onto the slide to prevent drying.

**Fluorescence Microscopy:**

- **Amytracker 480**: 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. For imaging with **Amytracker 480**, we recommend using the standard DAPI filter set.
- **Amytracker 520**: 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. For imaging with **Amytracker 520**, we recommend using the standard FITC or GFP filter set.
- **Amytracker 540**: 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. For imaging with **Amytracker 540**, we recommend using the standard FITC, GFP or YFP filter set.
- **Amytracker 630**: An excitation range of 458-514 nm and a detection range of 500-650 nm may be applied depending on available laser lines and filter sets. For imaging with **Amytracker 630**, we recommend using the standard PI, Cy3, TtRed, mCherry or Cy3.5 filter set.
- **Amytracker 680**: 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. For imaging with **Amytracker 680**, we recommend using the standard PI or Cy3.5 filter set.