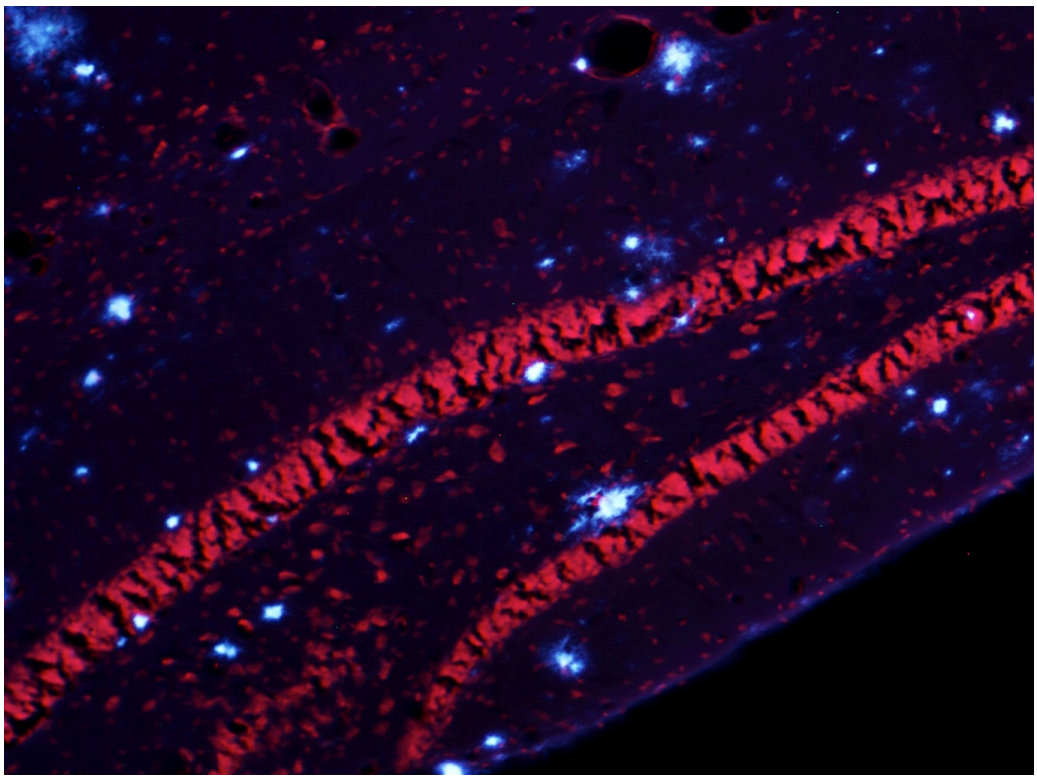
Amylo-Glo® RTD™ Amyloid Plaque Stain Reagent with EtBr Counter Stain Protocol

Catalog Number: TR-400-AG



For research use only, not for use in clinical and diagnostic procedures.

1. Description

The Biosensis TR-400-AG kit utilizes an ethidium bromide (EtBr) counter stain for a quick and effective way to visualize cell nuclei and cell bodies of cells, while under UV illumination allowing the assessment of amyloid plagues and cell/tissue positioning as well in one step.

Amylo-Glo® RTD™ "Ready-to-Dilute" Staining Reagent is designed to stain amyloid plaques in tissue sections. This novel marker has several advantages over other conventional markers such as Thioflavin S and Congo Red because of its unique chemical and spectral properties (Schmued L *et al.*, *J Neurosci Methods.* 2012 Jul 30;209(1):120-6). Using Amylo-Glo® results in a very bright blue UV excitable stain under physiological conditions that will not bleed through when illuminated with other filters. Its brightness makes it ideal for low magnification quantification studies, while its unique excitation and emission profile, and mild staining conditions, makes it ideal for combination in multiple immunofluorescent labeling studies.

Amylo-Glo® RTD™ is compatible with fresh, frozen, and formalin-fixed tissue and cells for immunohistochemistry or -cytochemistry, and it is particularly good for confocal and multiple labeling because of its high fluorescent intensity and high resistance to photo-bleaching. Moreover, because Amylo-Glo® fluoresces in the UV channel, double and triple labeling experiments can be performed very easily. To date it has been used to stain human, rodent, and even fish beta amyloid proteins successfully. See *Section 10* for guidance.

2. Fluorescent Imaging Settings

Amylo-Glo® RTD™ stained amyloid plaques can be visualized using UV epifluorescence illumination. Excitation is at 334 nm, emission when complexed to amyloid fibers is 438 nm with apparent shoulders at 421 nm and 530 nm.

Ethidium bromide has UV absorbance maxima at 300 and 360 nm, and an emission maximum at 590 nm and can thus be visualized under nearly identical filters as Amylo-Glo.

3. Materials Provided

1. One bottle containing 40 mL of 10X Amylo-Glo® RTD™ solution.
2. One bottle containing 40 mL of 10X Ethidium Bromide (EtBr) RTD™ solution.

4. Materials Required but not Supplied

- 0.9% saline solution, 1L (see Appendix A)
- Ethanol series: 100 mL each of 100%, 95% and 70% ethanol in water
- Tissue compatible slides, either gelatin-coated or treated for tissue adherence
- Slide warmer or air-dryer
- DPX mounting fluid, Vectashield, or other permanent mount, or glycerol solution for aqueous mounting, pH 6.0; check the pH, many fluorescent mounting medias are high pH, Amylo-Glo® requires pH below 7 for stability
- 100% Xylene solution

5. Reagent Preparation (Amylo-Glo®)

Prepare 1X Amylo-Glo® RTD™ by diluting the 10X stock 1:10. For example, to make 50 mL of 1X final solution for a standard Coplin jar, mix 5 mL of the dye stock solution into 45 mL of 0.9% saline solution. **Prepare just before use**. Keep both dye stock and particularly the diluted dye solution out of strong light. Storage of the diluted dye is not recommended for long periods. Use at room temperature.

6. Preparation of Tissue Slides

Freshly fixed tissue slices should be mounted onto either gelatin-coated slides or slides prepared for tissue adhesion, and the tissue fixed to the slides via air-drying at 50-60°C for at least 30 minutes prior to beginning the staining procedure. In general, charged slides alone are many times not sufficient to hold in place relatively thick fixed brain sections, so we recommend the use of charged, gelatin-coated slides if using thick sections. FFPE tissue sections can also be used. Standard deparaffinization protocols should be followed. Once rehydrated, the prepared slide are ready to begin the staining procedure.

7. Amyloid Plaque Staining Protocol

1. Dried, prepared slides are transferred into a 70% solution of ethanol for 5 minutes at room temperature.
2. The slides are then rinsed in distilled water (DW) for 2 minutes, without shaking.
3. The slides are then incubated for 10 minutes in the prepared 1X staining solution.
4. The slides are then rinsed in 0.9% saline solution for 5 minutes, without shaking.
5. The slides are then rinsed very briefly in fresh DW, approximately 15 seconds.

8. Counter Staining with EtBr

Prepare the 1X Ethidium bromide (EtBr) stain by diluting the Biosensis 10X EtBr RTD™ stock solution 1:10 with 0.9% saline solution.

1. The slide-mounted tissue sections are first stained with Amylo-Glo® RTD™, as described above up to step 5. Do not dry them.
2. The slides are then transferred directly into the prepared 1X EtBr RTD™ solution and incubated without shaking for 3 minutes.
3. The slides are then rinsed in 0.9% saline for 5 minutes.
4. Slides are then rinsed briefly (15 s) in distilled water.

At this stage the slides can be viewed directly after coverslipping with an aqueous mounting fluid that is pH 5.0-7.0 (**note**: high pH mounting fluids should not be used with Amylo-Glo® reagent).

Alternatively the slides can be either air-dried on a slide warmer (protected from light) until dry and dehydrated. Once dehydrated, the sections can be cleared by brief (~2 min) immersion in xylene and then coverslipped with DPX or VECTASHIELD Hard Set Mounting Medium for a more permanent slide if desired.

Amylo-Glo® RTD™ stained amyloid plaques can be visualized using UV epifluorescence illumination. Excitation is at 334 nm, emission when complexed to amyloid fibers is 438 nm with apparent shoulders at 421 nm and 530 nm. Ethidium bromide has UV absorbance maxima at 300 and 360 nm, and an emission maximum at 590 nm and can thus be visualized under nearly identical filters as Amylo-Glo.

9. Using Amylo-Glo® with EtBr Counter-stain and Antibodies

Amylo-Glo® RTD™ reagent is quite tolerant of a variety of staining and permeabilization methods. The key to success is to use neutral or slightly acidic conditions as the reagent's binding to the target is decreased in solutions with pH much above 8.0. The primary adverse effect of high pH would be the potential diffusion of the stain from its target resulting in a fuzzy appearance and lowered intensity.

To use our amyloid plaque reagent with antibodies, slide-mounted tissue sections are first stained with Amylo-Glo® RTD™ stain, as described in *Section 7*. The slides are then immediately used directly in antibody staining protocols followed by EtBr counterstaining as detailed in *Section 8*.

To prepare the Amylo-Glo® stained slides for immunohistochemistry, first rinse them for 2 minutes each in 3 changes of 0.1 M PBS, pH 7.2 in the dark, shielded from fluorescent light if possible. Then continue with a standard antibody staining protocol of your choice.

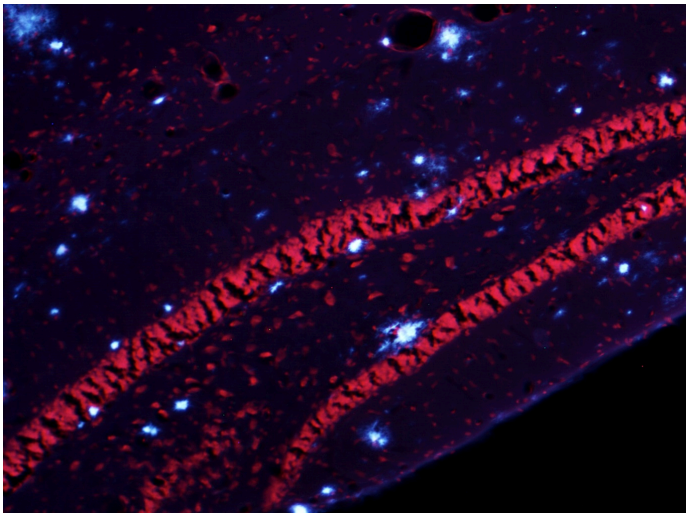
Please note: the dilutions, washes and incubations times given below are to be used as examples only, optimal conditions must be determined for each system by the investigator.

Example: Double-labeling with GFAP

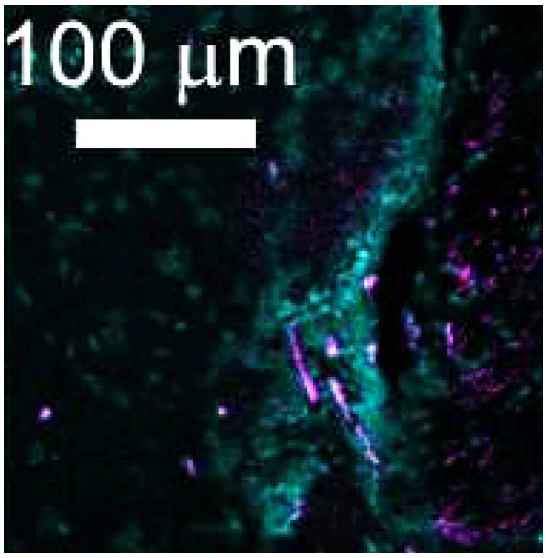
1. Prepared, Amylo-Glo® stained slides from *Section 7*, are incubated with 0.1M PBS containing 0.5% Triton-X100 and 1-10% blocking serum (in 0.1M PBS, pH 7.2) for 15 minutes. Permeabilization is necessary as GFAP is an internally localized cell protein.
2. The sections are then incubated with chicken anti-GFAP antibody (Biosensis catalog number C-1373-50) at a 1:2,000 dilution overnight in a humidity chamber at room temperature, in the dark.
3. The sections are then rinsed in 3 changes of PBS plus 0.5% Triton-X for 5 min per change.

4. Slides are then incubated with a biotinylated secondary donkey anti-chicken antibody, diluted 1:200 in PBS, for 2 hours at room temperature.
5. Sections are then rinsed three times for 5 minutes each with clean PBS.
6. The slides are then incubated in TRITC-labeled streptavidin (1:200) for 2 hours at room temperature, in the dark
7. The slides are then rinsed with 3 x 5 minute washes and prepared for EtBr counter-stain as detailed in *Section 8* above.

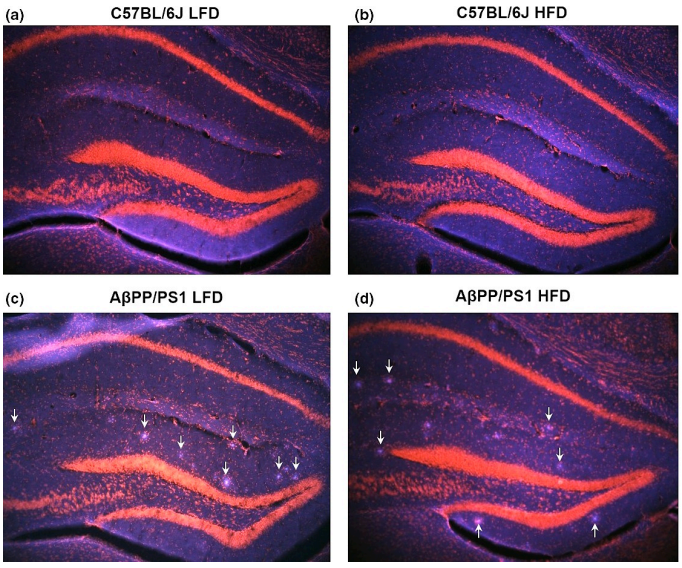
10. Example Images



Combined Amylo-Glo labeling of amyloid plaques (blue) with ethidium bromide Nissl (cell body) counterstain (red) in the dentate gyrus region of the hippocampus of the AD/Tg mouse. UV light excitation.



Amylo-Glo staining (pseudo colored pink) in the forebrain of Nothobranchius furzeri with EtBr counter-stain (blue-green). Taken from Matsui H *et al.*, *Cell Rep.* 2019 Feb 12;26(7):1727-1733.e6, Supplemental Information Figure S2.



Whole hippocampal representative images of amyloid plaque formation (blue) and nuclei staining (red) by EtBr in C57BL/6J LFD (a), C57BL/6J high-fat diet (HFD) (b), AbPP/PS1 LFD (c), and AbPP/PS1 HFD (d) mice at 109X magnification. Arrows indicate plaque formations in (c) and (d). Image taken from Hascup ER *et al.*, *J Neurochem.* 2019 Jan;148(2):219-237.

11. General References

Schmued L *et al.* (2012). Introducing Amylo-Glo, a novel fluorescent amyloid specific histochemical tracer especially suited for multiple labeling and large scale quantification studies. *J Neurosci Methods*. 209(1):120-6.

12. Specific References

Please refer to our website for the latest product-specific publications highlighting the use of Amylo-Glo® RTD™ Tracing Reagent with EtBr counter stain.

13. Related Products

TR-300-AG, Amylo-Glo® RTD™ Amyloid Plaque Stain Reagent.

Appendix A

Preparation of 0.9% saline solution (1 L)

- Weigh in 9 g of high grade sodium chloride (NaCl)
- Add NaCl to a clean volumetric flask or measuring cylinder
- Add 600 mL of distilled water and mix until dissolved
- Add distilled water to 1000 mL total volume and mix thoroughly
- For long-term storage, filter solution through a 0.22 µm membrane filter and transfer the solution to a sterile reagent bottle
- Label and date
- Store at 2-8°C for several months
- Equilibrate to room temperature before use