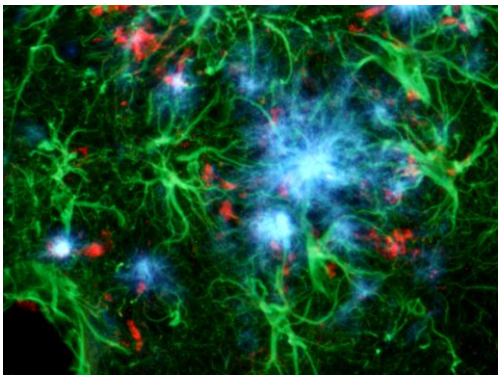
Amylo-Glo® RTD™ Amyloid Plaque Stain Reagent

Catalog number: TR-300-AG **Unit size:** 5 ml, 100X
Lot: see product label



This triple exposure allows for the simultaneous localization of Amylo-Glo® positive amyloid plaques (blue), GFAP positive hypertrophied astrocytes (green) and activated microglia (red) in the hippocampus of the AD/Tg mouse. Combined UV, blue and green light illumination.

Description: 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. (L. Schmued et al. (2012) *J.Neuroscience* Methods 209:120– 126). 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/emission profile and mild staining conditions makes it ideal for combination for multiple immunofluorescent labeling studies. Amylo-Glo® RTD™ is compatible with fresh, frozen, and formalin-fixed 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 (see protocol).

Materials provided:

1 bottle containing 5 mL of 100X Amylo-Glo® RTD™ (A-G RTD™) solution

Materials required but not supplied:

- 1 L of 0.9% normal saline (see preparation instructions in Appendix A)
- 100 mL 70% ethanol in water
- Ethanol series: 100 ml ea of 100%, 95% & 70% ethanol in water
- Tissue compatible slides, either gelatin coated or treated for tissue adherence
- Slide warmer or air-dryer
- DPX mounting fluid 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

Preparation of reagent:

Prepare 1X Biosensis Amylo-Glo® RTD™ (A-G RTD™) by diluting the 100X stock 1:100. For example to make 50mL of 1X final solution for a standard Coplin jar, use 0.5 ml of the dye stock solution into 49.5 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.

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 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.

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Basic Biosensis (A-G, RTD™) 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 (A-G, RTD™) 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 distilled water (DW), approximately 15 seconds.

At this stage the slides can be viewed directly after coverslipping with an aqueous mounting fluid that is pH 5-7.0. (NOTE: high pH mounting fluids should not be used with Amylo-Glo® reagents).

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 (e.g. 2 min) immersion in xylene and then coverslipped with DPX mounting media for a more permanent slide if desired.

Biosensis' (A-G) RTD™ stained amyloid plaques can be visualized using UV epifluorescent illumination. Excitation is at 334 nm, emission when complexed to amyloid fibers is 438 nm with apparent shoulders at 421 nm and 530 nm.

Additional procedures:

Double labeling: 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 the Biosensis (A-G) RTD™, as described above up to step 5. Do not dry them.
2. The slides are then transferred directly into the prepared Biosensis 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. Then rinsed briefly (15 s) in distilled water.

At this point the slides can be visualized or dried identically as directed above for A-G stain alone.

(Continued)

Using Biosensis (A-G) RTD™ stain with antibodies:

Biosensis (A-G) 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's 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 reagent with antibodies, slide mounted tissue sections are first stained with Biosensis (A-G) RTD™ plaque stain, as described in *Basic Biosensis (A-G, RTD™) staining protocol* to step 5.

At this stage the tissue slides are prepared for immunohistochemistry by first rinsing the stained slides for 2 minutes each in 3 changes of 0.1 M PBS, pH 7.2.

Double-labeling with GFAP: An example. *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.*

1. Prepared slides are incubated 0.1M PBS containing 0.5% Triton-X100 ™ and 1-10% blocking serum (in 0.1M PBS, pH 7.2) for 15 minutes.
2. The sections are then incubated with chicken anti-GFAP (Biosensis catalog number C-1373-50) antibody at a 1:2000 dilution overnight in a humidity chamber at room temperature.
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 in 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. Then the slides are incubated in TRITC labeled streptavidin (1:200) for 2 hours at room temperature.
7. The slides are then rinsed with three five minute washes, and dried on a slide warmer until completely dry.
8. Once dried the slides can be cleared with xylene and coverslipped with DPX mounting media and viewed.