FT-1B0250

Amylo-Glo

Product Description

Name :	**Amylo-Glo**
Catalog Number :	**FP-1B0250**, 5 ml solution at 100X
Structure & Properties:	MW= 392 g/mol
physical	
optical	**Absorption / Emission:** $\lambda_{exc}/\lambda_{em}$ (bound to amyloid) = 334/ 438 nm
Storage:	+4°C Protect from light and moisture.

Introduction

One of the hallmark pathologies associated with Alzheimer's disease (AD) is the conspicuous deposition of extracellular amyloid plaques within the forebrain. These plaques are primarily composed of fibrular aggregates of the A-beta peptide. Traditional methods for the histological localization of these plaques typically rely on the use of the tracers Congo Red or Thioflavin S. This study describes the characterization of a novel fluorescent histochemical probe, Amylo-Glo, for the high resolution and contrast localization of amyloid plaques in brain tissue sections. Potential advantages over conventional amyloid plaque stains such as Congo Red or Thioflavin S can be attributed to its unique chemical and spectral properties. Specifically, it 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 with multiple immunofluorescent labeling studies.

Directions for use

Protocol

1- Amylo-Glo staining: Tissue sections were first mounted onto gelatin (1% swine skin gelatin; 300 Bloom; Sigma Aldrich, St Louis, MO) coated slides and then air dried on a slide warmer at 50-60 degrees C for at least 30 min. The slides were then transferred to a 70% solution of ethanol for 5 min, followed by a 2 min rinse in distilled water (DW). The slides were then incubated for 10 min in the Amylo-Glo staining solution, which is made by diluting 1 ml of the 0.1% staining solution with 99 ml of 0.9% saline. The slides were then rinsed in saline for 5 min, followed by a brief (e.g., 15 s) rinse in DW. At this stage the slides were either air dried on a slide warmer or dehydrated via graduated alcohol solutions (e.g., 2 min in each of the following concentrations of ethanol: 70%, 95%, 100%, and 100%). Once dehydrated, the sections were cleared by brief (e.g. 2 min) immersion in xylene and then coverslipped with DPX mounting media.
2- Combined Amylo-Glo and Ethidium Bromide Counterstain: The slide mounted tissue sections were first stained with Amylo-Glo, as described above. The slides were then transferred directly into a solution of 0.0001% ethidium bromide in saline for 3 min. They were rinsed in saline for 5 min and then briefly (15 s) rinsed in distilled water. The sections were then dehydrated via graduated alcohol solutions, xylene cleared and coverslipped with DPX mounting media.

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3- Triple labeling - combine Amylo-Glo with GFAP and CD-68 immunohistochemistry: Slide mounted tissue sections were first stained with Amylo-Glo, as previously described. The slides were then transferred through 3 PBS rinses for 3 min each and then incubated in the same vehicle (PBS) plus 0.5% Triton-X for 30 min, followed by incubation in 10% normal horse serum for 15 min. The sections were then incubated for one day in a cocktail containing rabbit anti-GFAP antibody (DakoCytomation, Carpinteria, CA) at a dilution of 1:500 plus rat anti-CD68 antibody (Serotec, Raleigh, NC) at a dilution of 1:400. The sections were subsequently rinsed via three 3 min changes of PBS plus 0.5% Triton-X. The sections were then transferred for 2 h to a cocktail of secondary antibodies including a DyLight488 conjugated donkey anti-rabbit antibody and a biotinylated donkey anti-rat antibody (Jackson Immunoresearch, West Grove, PA), and were both used at a dilution of 1:200. Following 3 rinses in PBS plus 0.5% Triton-X for 3 min each, the sections were incubated for 1 h in TRITC-conjugated streptavidin (Jackson Immunoresearch, West Grove, PA) diluted 1:200 with vehicle. Finally, the sections were rinsed through 3 changes of PBS for 3 min per change, rinsed briefly (15 s) with distilled water, air dried on a slide warmer, briefly cleared in xylene and coverslipped with DPX.

Analysis

Amylo-Glo tissue was examined using an epifluoresent microscope with UV (Nikon UV-2A) filter cube. Excitation (325-375nm) Emission (400-450nm). It is not uncommon for Amylo-Glo to appear light yellow when examined by eye, yet appear a light blue color when photographed.

Technical and scientific information

References

- **Larry Schmued,** et al., Journal of Neuroscience Methods, Introducing Amylo-Glo, a novel fluorescent amyloid specific histochemical tracer especially suited for multiple labeling and large scale quantification studies 2009:120-126 (2012)

Related products

- Ethidium bromide, FP-06022B

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).