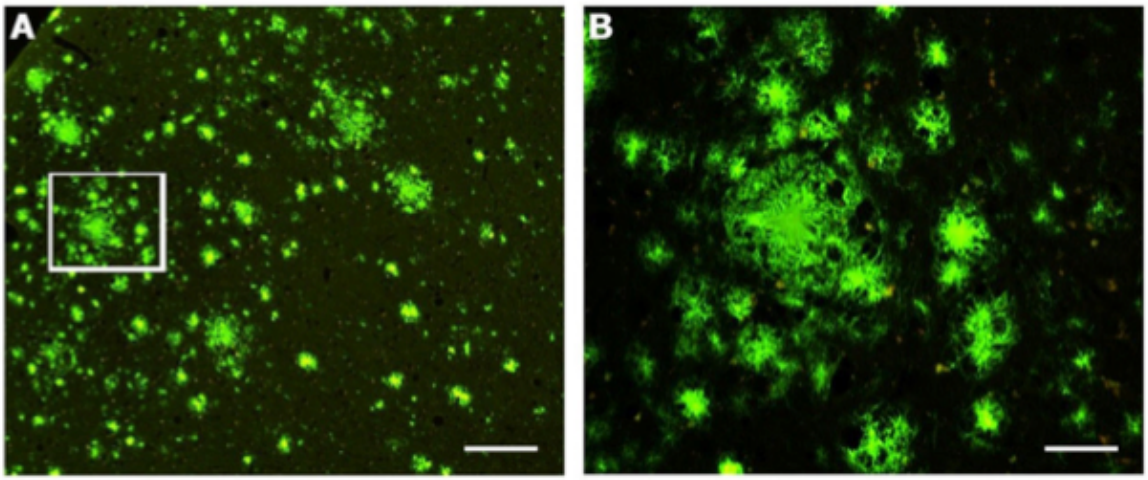
HQ-O RTD™ Amyloid Plaque Stain Reagent Protocol

Catalog Number: TR-700-HQO
TR-700-HQOT (trial size)



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

1. Description

HQ-O Ready-To-Dilute (RTD™) Stain Reagent is designed to label amyloid plaques in paraffin-embedded or freshly cut frozen tissue sections. As a fluorescent zinc chelator, HQ-O is unique as it takes advantage of the known presence of concentrated zinc in amyloid plaques. Studies with HQ-O revealed that fluorescent plaque-like structures are only seen when synthetic $A\beta_{x-42}$ is aggregated in the presence of zinc.

Under blue light excitation, plaque structures appear bright green fluorescent in the brain parenchyma, correlating closely with plaque structures observed following Aβ antibody staining.

HQ-O RTD™ staining reagent is compatible with other fluorophores, such as DAPI, Hoechst and ethidium bromide, as well as fluorescent-labelled antibodies with emission spectra in the blue and/or red emission range of fluorescent microscopes.

Due to its zinc-chelating characteristics, HQ-O RTD™ staining reagent may visualize globular structures within blood vessels and intravascular leucocytes.

HQ-O RTD™ staining reagent has multiple advantages over older blue-light exciting stains such as Thioflavin S. Thioflavin S typically exhibits relatively low contrast and resolution and suffers from bleed-through when excited by wavelengths other than blue light. HQ-O RTD™ staining reagent suffers none of these setbacks and not only provides a higher contrast and longer lasting dye, but because it lacks excitation bleed-through, HQ-O can be readily adapted to multiple labelling studies very easily.

2. Fluorescent Imaging Settings

To visualize the HQ-O tracer, it is recommended to use a filter cube designed for visualizing Fluorescein/FITC or a blue-light laser. Although it can be seen with both narrow and wide-band pass filters, there is no need to use a narrow band filter since the compound does not “bleed through” when excited with other filters. A recommended excitation range of a wide band filter is 447 – 503 nm, with a peak at 475. Note that it is this range and filter peak that has been used to generate the photographs in this insert.

3. Materials Provided

TR-700-HQOT (trial-size): One bottle containing 20 mL of 10X HQ-O RTD™ solution This quantity will be sufficient for approximately 4 Coplin Jars or 1-2 staining dishes.

TR-700-HQO : One bottle containing 40 mL of 10X HQ-O RTD™ solution. This quantity will be sufficient for approximately 8 Coplin Jars or 2-4 staining dishes.

4. Materials Required but not Supplied

- 0.9% saline solution, 200 mL (see Appendix A)
- 100% ethanol, 100 mL
- 70% ethanol in water, 100 mL
- Distilled water
- 100% xylene solution
- DPX mounting medium, or other non-polar and non-fluorescent mounting medium

5. Reagent Preparation

Note: Prepare 1X dye working dilutions just before use!

Prepare a 1X working solution by diluting the 10X stock solution with 0.9% saline solution. For example, to make 100 mL of final 1X working solution, mix 10 mL of 10X stock solution with 90 mL saline solution in a clean container. Mix thoroughly and keep diluted dye in the dark at room temperature.

The 1X staining solution should be used within 24 hours. Long-term storage of diluted dye is not recommended.

6. Preparation of Tissue Slides

Frozen sections are mounted onto gelatin-coated slides and allowed to dry on a slide warmer for at least 20 min. Typically, the slides are first immersed in 100% ethanol for 5 min followed by 3 min in 70% ethanol and 3 min in distilled water. Should exposure to solvents be counter-indicated, this step may be omitted, and the sections may simply be rehydrated in distilled water for 3 min.

FFPE tissue sections can also be used. Paraffin-embedded sections are first deparaffinized in xylene and then rehydrated with a graded series of alcohols and finally in distilled water.

7. Amyloid Plaque Staining Protocol

Note: Perform tissue staining in the dark!

Frozen Sections

The slides are transferred to the HQ-O staining solution. The optimal staining time is temperature dependent, therefore overnight (16-24 hours) incubation is required when staining fresh tissue sections at room temperature, while only 3 hours incubation time is required at 60°C.

Paraffin-Processed Sections

The hydrated slides are transferred to the HQ-O staining solution. At room temperature, the paraffin-processed tissue will require around 3 hours to fully stain, while tissue sections incubated at 60°C will require only about 45 min to stain.

Slide Clearing and Mounting

Following staining, all slides are rinsed for 3 min through 2 changes of distilled water and then allowed to air dry on a slide warmer. Slides are cleared by brief (1-2 min) immersion in xylene and then coverslipped with DPX or other non-polar and non-fluorescent mounting media.

Multiple Labeling Studies

HQ-O staining lends itself to multiple labeling studies and in such studies, the tissue is first stained with HQ-O, as indicated above, and then double-labeled with a variety of labelling reagents from antibodies to dyes and other stains.

8. Co-Labelling Experiments with Fluorescent Antibodies

Important Notes:

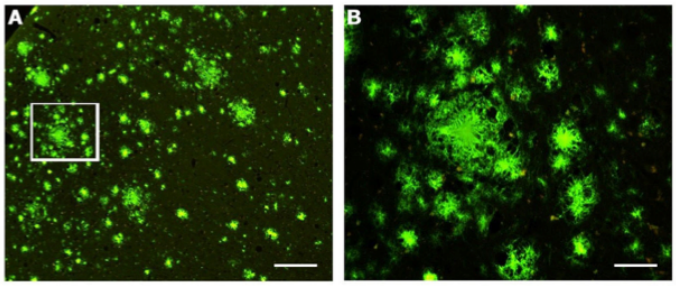
(1) Antibody staining is typically performed **after staining with HQ-O** (Section 7), and before slide clearing and mounting.
(2) The basic antibody staining protocol here will need to be optimized by the end-user for incubation times, washing, dilution factors and blocking conditions.
(3) The primary antibody must be chosen for its intended application, that is immuno-fluorescence staining of frozen or FFPE sections, as some antibodies may work on one, but not the other.
(4) The fluorescent antibody tag must be chosen to not interfere with blue light illumination of HQ-O and green filter emission.
(5) During antibody staining, the slide should be kept in a humidity chamber

Protocol:

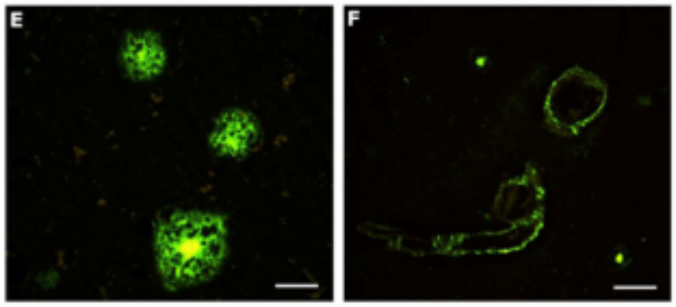
1. Rinse the HQ-O stained slides in 0.1 M PBS, pH 7.2-7.6, 2 minutes each for a total of 3 times
2. Add blocking solution consisting of 0.1 M PBS, pH 7.2-7.6 with 0.5% Triton X-100 and 1-10% normal serum from the animal species the secondary antibody was raised in (eg., horse serum if secondary antibody has been raised in donkey)
3. Block sections for 20 minutes
4. Rinse sections with 0.1 M PBS, pH 7.2-7.6 (3 x 2 min)
5. Incubate sections overnight at room temperature with primary antibody at a dilution recommended by the manufacturer, diluted in 0.1 M PBS, pH 7.2-7.6, containing 0.5% Triton X-100 and 2% normal serum (same serum as blocking solution)
6. Rinse sections with 0.1 M PBS, pH 7.2-7.6 (3 x 2 min)
7. Incubate sections for 2 hours at room temperature with biotinylated secondary antibody, raised against the host of the primary antibody, at a dilution recommended by the manufacturer, diluted in 0.1 M PBS, pH 7.2-7.6, containing 0.5% Triton X-100 and 2% normal serum (same serum as blocking solution)
8. Rinse sections with 0.1 M PBS, pH 7.2-7.6 (3 x 2 min)
9. Incubate sections for 2 hours at room temperature with fluorescent-labelled streptavidin or avidin complex, at a dilution recommended by the manufacturer, diluted in 0.1 M PBS, pH 7.2-7.6, containing 0.5% Triton X-100 and 2% normal serum (same serum as blocking solution)
10. Rinse sections with 0.1 M PBS, pH 7.2-7.6 (5 x 2 min)

11. Rinse sections in distilled water (3 x 2 min), air-dry, and clear by brief (1-2 min) immersion in xylene and then coverslip with DPX or other non-polar and non-fluorescent mounting media.

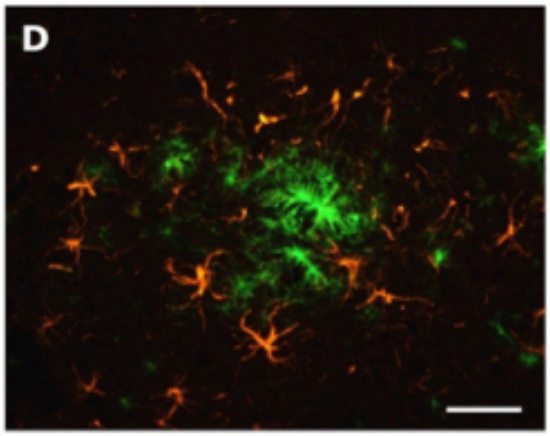
9. Example Images



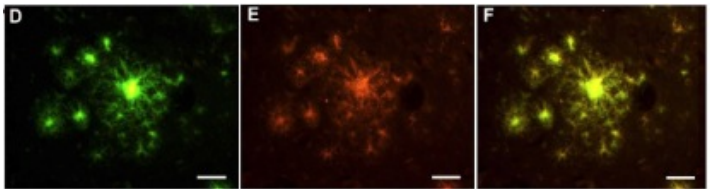
Labelling of amyloid plaques (green) in paraffin-processed cortex from a 1 year old APP/PSI mouse model of AD (**A**). **B**: High-magnification view of the cortical region outlined in white in A.



Three amyloid plaques (green) are seen in the cortex of a human with Alzheimer's disease (**E**). **F**: An example of vascular plaques in the cortex of a human AD patient.



Within the CA4 region of the hippocampus, co-labeling using GFAP antibody demonstrates the relationship between hypertrophied astrocytes (orange) and the amyloid plaques (green) that they typically surround. GFAP-immunoreactivity was visualized with TRITC fluorophore.



HQ-O stained plaques can be seen within the CA-1 region of the hippocampus (**D**). This same field of view stained with immunofluorescent methods using the Aβx-40/42 MOAB-2 antibody, **M-1586-100** Biosensis antibody reveals a similar but somewhat more restricted labeling of the same amyloid plaques (**E**), which is apparent when the images are merged (**F**).

Photo taken with permission from Current Alzheimer Research, 2019, Vol. 16, No. 7 583, Figure 4.

Appendix A

Preparation of 0.9% saline solution (200 mL)

- Weigh in 1.8 g of high grade sodium chloride (NaCl)
- Add NaCl to a clean volumetric flask or measuring cylinder
- Add 150 mL of distilled water and mix until dissolved
- Add distilled water to 200 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