Immunohistochemistry Protocol for Beta Amyloid Products using USA Detection Kit V.2

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Guidelines

Protocol can be used for Beta Amyloid products that list “IHC” as an application on the datasheet (*e.g.* clones 4G8, 6E10, etc).

Use with Ultra Streptavidin Detection Kit (**BioLegend Cat #929501**/SIG-32250) or (**BioLegend Cat #929401**/SIG-32248). All steps should be done in a humidity chamber such as **BioLegend Cat #926301**/SIG-31031.

Materials

MATERIALS

- Ultra Streptavidin (USA) HRP Detection Kit (Multi-Species DAB) (Previously Covance catalog# SIG-322 **BioLegend Catalog #**929501
- Ultra Streptavidin (USA) HRP Detection Kit (Multi-Species AEC) (Previously Covance catalog# SIG-322 **BioLegend Catalog #**929401
- Humidity Chamber Plus (Previously Covance catalog# SIG-31031) **BioLegend Catalog #**926301

1 Clear Slides: Remove paraffin and hydrate the tissue
Note: If using frozen sections, allow slides to come to room temperature for 15 minutes & proceed to step (F) only

- A. Xylene - 5 minutes in each of (3) different 250 mL containers
- B. 100% alcohol - 5 minutes in each of (3) different 250 mL containers
- C. 95% alcohol - 3 minutes in (1) 250 mL container
- D. 70% alcohol - 3 minutes in (1) 250 mL container
- E. Water - 1 minutes in each of (3) different 250 mL containers
- F. H_2O_2 (3%) - 15 minutes in (1) 250 mL container

2 Rinse slides with lab grade water.
Note: Lab grade filtered water such as injection grade, cell culture grade, Reverse Osmosis De-Ionization (RODI).

3 Antigen Retrieval (refer to product datasheet, not always required)

3.1 70% Formic Acid - incubate the slides for 20 minutes at room temperature.
Note: This antigen retrieval step is harsh on the tissue. If using frozen sections reduce time to 5-10 minutes or omit if tissue falls off the slide.

3.2 Rinse Slides with 1X PBS.

4 Apply serum block for at least 5 minutes. **Do not wash** after this step.

5 Blot off serum block.

6 Apply primary antibody - dilute to 1 µg/mL in PBS.

7 Incubate primary antibody 60 minutes at room temperature.

8 Rinse slides with 1X PBS.

9 Apply USA Linking reagent - 20 minutes incubation.

10 Rinse slides with 1X PBS.

11 Apply Labeling Reagent - 20 minutes incubation.

12 Rinse with 1X PBS.

13 Apply chromogen - 5 minutes incubation. Dilute according to manufacturer's instructions.

13.1 AEC Chromogen: 20 µL AEC chromogen + 1 mL AEC substrate buffer.

13.2 DAB Chromogen: 40 µL DAB chromogen + 1 mL DAB substrate buffer.

14 Rinse slides with lab grade water.

15 Counterstain

15.1 Submerge slides in Mayer’s Hematoxylin for 30 seconds.

15.2 Rinse under running lab grade water for 1 minute or until water is clear.

15.3 Submerge slides in Bluing Reagent for 1 minute.

15.4 Rinse under running lab grade water for 1 minute.

16 Clear slides: Dehydrate the tissue.

16.1 95% alcohol 3 minutes in (1) 250 mL container.

16.2 100% alcohol 5 minutes in each of (3) different 250 mL container.

16.3 Xylene 5 minutes in each of (3) different 250 mL container.

17 Cover slip slide using Permanent Aqueous Mounting Medium.
Note: Do not use xylene based mount with AEC Chromogen as it will dissolve the chromogen.