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

DOI
dx.doi.org/10.17504/protocols.io.8×4hxqw

Sam Li[1]

[1]BioLegend

BioLegend
Tech. support email: **tech@biolegend.com**

Sam Li
BioLegend

DOI: https://dx.doi.org/10.17504/protocols.io.8x4hxqw

External link: https://www.biolegend.com/protocols/immunohistochemistry-protocol-for-beta-amyloid-products-using-usa-detection-kit/4259/

Protocol Citation: Sam Li . Immunohistochemistry Protocol for Beta Amyloid Products using USA Detection Kit. **protocols.io https://dx.doi.org/10.17504/protocols.io.8x4hxqw**

Created: November 02, 2019

Last Modified: November 02, 2019

Protocol Integer ID: 29404

Keywords: IHC, beta amyloid, immunohistochemistry protocol for beta amyloid product, beta amyloid product, immunohistochemistry protocol, using usa detection kit, usa detection kit

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

Troubleshooting

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.