Immunohistochemistry Protocol for Beta Amyloid Products using USA Detection Kit

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Abstract

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Guidelines

-Protocol can be used for Beta Amyloid products that list “IHC” as an application on the datasheet (ex 4G8, 6E10, etc)

-Use with Ultra Streptavidin Detection Kit (SIG-32250) or (SIG-32248). All steps should be done in a humidity chamber such as SIG-31031

Protocol

Clear Slides

Step 1.

Clear Slides: Removes paraffin and hydrates 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 250mL containers
B.	100% alcohol	5 minutes in each of (3) different 250mL containers
C.	95% alcohol	3 minutes in (1) 250mL container
D.	70% alcohol	3 minutes in (1) 250mL container
E.	water	1 minutes in each of (3) different 250mL containers

F.	H_2O_2 (3%)	15 minutes	in (1) 250mL container

Rinse Slides

Step 2.

Rinse slides with lab grade water.

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

Antigen Retrieval

Step 3.

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

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

DURATION
00:20:00

Antigen Retrieval

Step 4.

Rinse Slides with 1X PBS

Step 5.

Apply serum block for at least 5 minutes.

Do NOT wash after this step

DURATION
00:05:00

Step 6.

Blot off serum block

Step 7.

Apply primary antibody – dilute to 1 mg/mL in PBS

Step 8.

Incubate primary antibody 60 minutes at room temperature

DURATION
01:00:00

Step 9.

Rinse slides with 1X PBS

Step 10.

Apply USA Linking reagent - 20 minutes incubation

DURATION
00:20:00

Step 11.

Rinse slides with 1X PBS

Step 12.

Apply Labeling Reagent – 20 minutes incubation

DURATION
00:20:00

Step 13.

Rinse with 1X PBS

Step 14.

Apply chromogen – 5 minutes incubation.

Dilute according to manufacturer's instructions

1. AEC Chromogen: 20mL AEC chromogen + 1mL AEC substrate buffer
2. DAB Chromogen: 40 mL DAB chromogen + 1mL DAB substrate buffer

DURATION
00:05:00

Step 15.

Rinse slides with lab grade water

Counterstain

Step 16.

Submerge slides in Mayer’s Hematoxylin for 30 seconds

DURATION
00:00:30

Counterstain

Step 17.

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

DURATION
00:01:00

Counterstain

Step 18.

Submerge slides in Bluing Reagent for 1 minute

DURATION
00:01:00

Counterstain

Step 19.

Rinse under running lab grade water for 1 minute

DURATION
00:01:00

Clear slides

Step 20.

Clear slides: Dehydrate the tissue.

1. 95% alcohol 3 minutes in (1) 250mL container
2. 100% alcohol 5 minutes in each of (3) different 250mL container
3. Xylene 5 minutes in each of (3) different 250mL container

Coverslip

Step 21.

Cover slip slide using Permanent Aqueous Mounting Medium (SIG-31010).

Note: Do not use xylene based mount with AEC Chromogen as it will dissolve the chromogen