Immunohistochemistry Protocol for Sternberger Monoclonal Antibodies

Kelsey Miller

Abstract

Citation: Kelsey Miller Immunohistochemistry Protocol for Sternberger Monoclonal Antibodies. **protocols.io** dx.doi.org/10.17504/protocols.io.e2jbgcn
Published: 03 Jun 2016

Guidelines

Use with Ultra Streptavidin Detection Kit (SIG-32250) or (SIG-32248)

Positive control: Normal human cerebellum (except SMI-71, which should be rat brain)

Protocol

Clear Slides

Step 1.

Clear Slides: Removes paraffin and hydrates the tissue.

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.	H2O2 (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.

Heat slides in 1X Retrieve ALL3 solution for 1 minute 40 seconds on high power in microwave

DURATION
00:01:40

Antigen Retrieval

Step 4.

Reduce to low power and simmer 10 minutes in microwave

DURATION
00:10:00

Antigen Retrieval

Step 5.

Remove from microwave and allow slides to cool on the bench top for 10 minutes

DURATION
00:10:00

Antigen Retrieval

Step 6.

Rinse slides with lab grade water

Step 7.

Apply serum block for at least 5 minutes. Do NOT wash after this step

DURATION
00:05:00

Step 8.

Blot off serum block

Step 9.

Apply primary antibody (see recommended dilution from datasheet)

Step 10.

Incubate primary antibody 60 minutes at room temperature.

DURATION
01:00:00

Step 11.

Rinse slides with 1X PBS

Step 12.

Apply USA Linking reagent - 20 minutes incubation.

(If using a biotinylated product, omit this step)

DURATION
00:20:00

Step 13.

Rinse slides with 1X PBS

Step 14.

Apply Labeling Reagent – 20 minutes incubation

DURATION
00:20:00

Step 15.

Rinse with 1X PBS

Step 16.

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

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

DURATION
00:05:00

Step 17.

Rinse slides with lab grade water

Counterstain

Step 18.

Submerge slides in Mayer's Hematoxylin for 30 seconds

DURATION
00:00:30

Counterstain

Step 19.

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

DURATION
00:01:00

Counterstain

Step 20.

Submerge slides in Bluing Reagent for 1 minute

DURATION
00:01:00

Counterstain

Step 21.

Rinse under running lab grade water for 1 minute

DURATION
00:01:00

Clear slides

Step 22.

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 23.

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