Immunohistochemistry Protocol for Sternberger Monoclonal Antibodies V.2

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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)

Clear Slides

1 Clear Slides: Removes paraffin and hydrates the tissue.

	Xylene	5 minutes in each of (3) different 250mL containers
	100% alcohol	5 minutes in each of (3) different 250mL containers
	95% alcohol	3 minutes in (1) 250mL container
	70% alcohol	3 minutes in (1) 250mL container
	water	1 minutes in each of (3) different 250mL containers
	H2O2 (3%)	15 minutes in (1) 250mL container

Rinse slides

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

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

00:01:40

4 Reduce to low power and simmer 10 minutes in microwave.

00:10:00

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

00:10:00

6 Rinse slides with lab grade water.

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

00:05:00

8 Blot off serum block.

9 Apply primary antibody (see recommended dilution from datasheet).

10 Incubate primary antibody 60 minutes at room temperature.
01:00:00

11 Rinse slides with 1X PBS.

12 Apply USA Linking reagent - 20 minutes incubation.
(If using a biotinylated product, omit this step)
00:20:00

13 Rinse slides with 1X PBS.

14 Apply Labeling Reagent - 20 minutes incubation. 00:20:00

15 Rinse with 1X PBS.

16 Apply chromogen - 5 minutes incubation. Dilute according to manufacturer's instructions.
00:05:00

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

Counterstain

17 Submerge slides in Mayer's Hematoxylin for 30 seconds.
00:00:30

18 Rinse under running lab grade water for 1 minute or until water is clear.
00:01:00

19 Submerge slides in Bluing Reagent for 1 minute.
00:01:00

20 Rinse under running lab grade water for 1 minute.
00:01:00

Clear slides

21 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

22 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