Dec 05, 2019

Immunohistochemistry Protocol for Keratin Antibodies V.3

Sam Li[1]

[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.95ph85n

BioLegend

Sam Li
BioLegend

EXTERNAL LINK

https://www.biolegend.com/protocols/immunohistochemistry-protocol-for-keratin-antibodies/4287/

GUIDELINES

Use with Ultra Streptavidin Detection Kit (BioLegend Cat # 929501) or (BioLegend Cat # 929401)

Positive control: Normal human skin

FAQ:

Do I need to perform antigen retrieval on my formalin-fixed, paraffin-embedded samples prior to staining?

- In most cases, this is true. Antigen retrieval helps both the accessibility of the antibody to the tissue and also counteracts the fixation effects on the recognized epitopes. Check the application references for use of the antibody in paraffin-embedded samples.

Can antibody X be used for immunohistochemistry? What concentration do I use?

- Typical concentrations of monoclonal antibodies for use in IHC are from 5-25 µg/ml. Polyclonal antibodies can be used at a range of 1-10 µg/ml. While we do not test for IHC application in house, we will indicate on the datasheet if an antibody has been published for use in this application. In addition, you can do a literature search with the clone name and immunohistochemistry/paraffin/frozen to see what the protocol details are.

MATERIALS

NAME	CATALOG #	VENDOR
Ultra Streptavidin (USA) HRP Detection Kit (Multi-Species DAB) (Previously Covance catalog# SIG-322	929501	BioLegend
Ultra Streptavidin (USA) HRP Detection Kit (Multi-Species AEC) (Previously Covance catalog# SIG-322	929401	BioLegend

Clear Slides: Removes paraffin and hydrates the tissue

1 **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 **00:05:00**

B. 100% alcohol
5 minutes in each of (3) different 250mL containers **00:05:00**

C. 95% alcohol
3 minutes in (1) 250mL container **00:03:00**

D. 70% alcohol
3 minutes in (1) 250mL container **00:03:00**

E. Water
1 minutes in each of (3) different 250mL containers **00:01:00**

F. H_2O_2 (3%)
15 minutes in (1) 250mL container **00:15:00**

Rinse Slides

2 Rinse slides with lab grade water.

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

Antigen Retrieval

3 **A**. 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.
B. Rinse Slides with 1X PBS.
C. Remove from microwave and allow slides to cool on the bench top for 10 minutes.
D. Rinse slides with lab grade water.

4 Apply serum block for at least 5 minutes.
Do not wash after this step.
00:05:00

5 Blot off serum block

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

7 Incubate primary antibody 60 minutes at room temperature.

8 Rinse slides with 1X PBS.

9 Apply USA Linking reagent - 20 minutes incubation. **00:20:00**

10 Rinse slides with 1X PBS.

11 Apply Labeling Reagent - 20 minutes incubation **00:20:00**

12 Rinse with 1X PBS.

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

A. AEC Chromogen: 20µL AEC chromogen + 1mL AEC substrate buffer
00:05:00

14 Rinse slides with lab grade water.

Coverslip

15 A. Submerge slides in Mayer's Hematoxylin for 30 seconds
00:00:30
B. Rinse under running lab grade water for 1 minute or until water is clear.
C. Submerge slides in Bluing Reagent for 1 minute.
D. Rinse under running lab grade water for 1 minute.
E. Cover slip slide using Permanent Aqueous Mounting Medium.

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