



Immunohistochemistry Protocol for Keratin Antibodies [↗](#)

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¹BioLegend

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Version 2

BioLegend

Working



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BioLegend



EXTERNAL LINK

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

PROTOCOL STATUS

Working

GUIDELINES

- Use with Ultra Streptavidin Detection Kit (SIG-32250) or (SIG-32248)
- Positive control: Normal human skin

Clear Slides: Removes paraffin and hydrates the tissue

1

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. H₂O₂ (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 Submerge slides in Mayer's Hematoxylin for 30 seconds

🕒 00:00:30

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

🕒 00:01:00

- 17 Submerge slides in Bluing Reagent for 1 minute

🕒 00:01:00

- 18 Rinse under running lab grade water for 1 minute

🕒 00:01:00

- 19 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.



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