Immunohistochemistry Protocol for Keratin Antibodies V.2

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GUIDELINES

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

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External link:

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

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Clear Slides: Removes paraffin and hydrates the tissue

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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: 20 μ L 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.