# 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

## 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. $H_2O_2$ (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 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.*