Immunohistochemistry Protocol for Keratin Antibodies

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Abstract

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Guidelines

- Use with Ultra Streptavidin Detection Kit (SIG-32250) or (SIG-32248)

- Positive control: Normal human skin

Protocol

Clear Slides

Step 1.

Clear Slides: Removes paraffin and hydrates the tissue.

A. Xylene:
5 minutes in each of (3) different 250mL containers

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

C. 95% alcohol
3 minutes in (1) 250mL container

D. 70% alcohol
3 minutes in (1) 250mL container

E. Water
1 minutes in each of (3) different 250mL containers

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

Rinse Slides

Step 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

Step 3.

Heat slides in 1X Sodium Citrate solution for 1 minute 25 seconds on high power in microwave.

DURATION
00:01:25

Antigen Retrieval

Step 4.

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

DURATION
00:10:00

Antigen Retrieval

Step 5.

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

DURATION
00:10:00

Antigen Retrieval

Step 6.

Rinse slides with lab grade water.

Step 7.

Apply serum block for at least 5 minutes.

Do NOT wash after this step

DURATION
00:05:00

Step 8.

Blot off serum block

Step 9.

Apply primary antibody (see recommended dilution from datasheet)

Step 10.

Incubate primary antibody 60 minutes at room temperature

DURATION
01:00:00

Step 11.

Rinse slides with 1X PBS

Step 12.

Apply USA Linking reagent - 20 minutes incubation

DURATION
00:20:00

Step 13.

Rinse slides with 1X PBS

Step 14.

Apply Labeling Reagent – 20 minutes incubation

DURATION
00:20:00

Step 15.

Rinsewith 1X PBS

Step 16.

Apply chromogen – 5 minutes incubation.

Dilute according to manufacturer’s instructions.

AEC Chromogen: 20µL AEC chromogen + 1mL AEC substrate buffer

DURATION
00:05:00

Step 17.

Rinse slides with lab grade water

Coverslip

Step 18.

Submerge slides in Mayer's Hematoxylin for 30 seconds

DURATION
00:00:30

Coverslip

Step 19.

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

DURATION
00:01:00

Coverslip

Step 20.

Submerge slides in Bluing Reagent for 1 minute

DURATION
00:01:00

Coverslip

Step 21.

Rinse under running lab grade water for 1 minute

DURATION
00:01:00

Coverslip

Step 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.