Immunohistochemistry Protocol for Ultra Streptavidin Detection Kits (USA)

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Abstract

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Guidelines

Use with all Ultra Streptavidin Detection Kits.

All steps should be done in a humidity chamber such as 926301 (http://www.biolegend.com/humidity-chamber-plus-11211.html)

Protocol

Clear Slides

Step 1.

Clear Slides: Removes paraffin and hydrates the tissue.

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

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. H2O2 (3%)
15 minutes in (1) 250mL container (927401, 927402, or use #1 Blocking Reagent if included in kit)

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-Ionization (RODI).

Antigen Retrieval

Step 3.

Antigen Retrieval (refer to product datasheet, not always required)

Retrieve-ALL (927901, 928201, 927601) or Sodium Citrate (928501, 928601)

Heat slides in microwave on high for 1 minute 40 seconds in the appropriate retrieval solution at 1X

DURATION
00:01:40

Antigen Retrieval

Step 4.

Reduce to low power and simmer 10 minutes in the microwave

DURATION
00:10:00

Antigen Retrieval

Step 5.

Allow to cool on the bench top for 10 minutes

DURATION
00:10:00

Antigen Retrieval

Step 6.

Rinse with lab grade water

Antigen Retrieval

Step 7.

Rinse Slides with 1X PBS (926201)

Note: Other antigen retrievals could include EDTA, Proteinase K, Pepsin, protease VIII – follow antibody manufacturer's instructions

Step 8.

Apply blue serum block for at least 5 minutes at room temperature.

Do NOT wash after this step.

DURATION
00:05:00

Step 9.

Blot off serum block

Apply primary antibody

Step 10.

6 mL predilute antibodies are ready to use, do not dilute

1 mL concentrate products can be diluted >1:40 in PBS or other antibody diluent

If using a non-BioLegend antibody, dilute according to the manufacturer's instructions

Step 11.

Incubate primary antibody 20-60 minutes at room temperature (refer to incubation time listed on the datasheet).

Step 12.

Rinse slides with 1X PBS.

Step 13.

Apply yellow USA Linking Reagent – and incubate slides for 20 minutes at room temperature

DURATION
00:20:00

Step 14.

Rinse slides with 1X PBS

Step 15.

Apply orange USA Labeling Reagent – and incubate slides for 20 minutes at room temperature

DURATION
00:20:00

Step 16.

Rinse with 1X PBS.

Step 17.

Apply chromogen and incubate slides for 5 minutes at room temperature.

A. AEC Chromogen: 20µL AEC chromogen + 1mL AEC substrate buffer (1:50 Dilution)

B. DAB Chromogen: 40 µL DAB chromogen + 1mL DAB substrate buffer (1:25 Dilution)

Note: Not all USA Kits contain chromogen. If using a non-BioLegend chromogen, dilute and incubate according
to the manufacturer's instructions

DURATION
00:05:00

Step 18.

Rinse slides with lab grade water

Counterstain

Step 19.

Submerge slides in Hematoxylin for 30 seconds (not provided)

DURATION
00:00:30

Counterstain

Step 20.

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

DURATION
00:01:00

Counterstain

Step 21.

Submerge slides in Bluing Reagent for 1 minute (not provided)

DURATION
00:01:00

Counterstain

Step 22.

Rinse under running lab grade water for 1 minute

DURATION
00:01:00

Clear slides

Step 23.

Clear slides: Dehydrate the tissue.

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

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

C. Xylene 5 minutes in each of (3) different 250mL container

Coverslip

Step 24.

Cover slip slide using Permanent Aqueous Mounting Medium (926101) or Xylene Based medium.

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