Immunohistochemistry Protocol for Ultra Streptavidin Detection Kits (USA) V.3

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Guidelines

Use with all Ultra Streptavidin Detection Kits.

All steps should be done in a humidity chamber such as **926301**.

General Tips & FAQ

Tips:

- The multi-species system is suitable for use with primary antibodies that were raised in either mice or rabbits.
- The murine system is suitable for use with primary antibodies that were raised in mice.
- The rabbit system is suitable for use with primary antibodies that were raised in rabbits.
- Chromogen may be purchased separately.

1 Clear Slides: Remove paraffin and hydrate the tissue.
Note: If using frozen sections, allow slides to come to room temperature for 15 minutes & proceed to step (F) only

1. Xylene - 5 minutes in each of (3) different 250 mL containers
2. 100% alcohol - 5 minutes in each of (3) different 250 mL containers
3. 95% alcohol - 3 minutes in (1) 250 mL container
4. 70% alcohol - 3 minutes in (1) 250 mL container
5. Water -1 minutes in each of (3) different 250 mL containers
6. #1 Blocking Reagent - 15 minutes using # 1 Blocking Reagent (clear in color) included in 50 test kit only, or **927401**/**927402** (sold separately) for 150 and 500 test kits.

2 Rinse slides with lab grade water.
Note: Lab grade filtered water such as injection grade, cell culture grade, Reverse Osmosis De-Ionization (RODI).

3 Antigen Retrieval - Retrieve-ALL (**927901**,**928201**,**927601**) or Sodium Citrate (**928502**)

1. Heat slides in microwave on high for 1 minute 40 seconds in the appropriate retrieval solution at 1X.
2. Reduce to low power and simmer 10 minutes in the microwave.
3. Allow to cool on the bench top for 10 minutes.
4. Rinse with lab grade water.

4 Antigen Retrieval - Rinse Slides with 1X PBS (**926201**)
Note: Other antigen retrievals could include EDTA, Proteinase K, Pepsin, protease VIII - follow antibody manufacturer's instructions.

5 Apply #2 Blocking Reagent (blue in color) for at least 5 minutes at room temperature. Do NOT wash after this step only.

6 Blot off serum block.

7 Apply primary antibody.

1. 6 mL predilute antibodies are ready to use, do not dilute.
2. 1 mL concentrate products can be diluted > 1:40 in PBS or other antibody diluent.
3. If using a non-BioLegend antibody, dilute according to the manufacturer's instructions.

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

9 Rinse slides with 1X PBS.

10 Apply #4 Linking Reagent (yellow in color) - and incubate slides for 20 minutes at room temperature.

11 Rinse slides with 1X PBS.

12 Apply #5 Labeling Reagent (orange in color) - and incubate slides for 20 minutes at room temperature.

13 Rinse with 1X PBS.

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

1. AEC Chromogen: 20 µL AEC chromogen + 1 mL AEC substrate buffer (1:50 Dilution)
2. DAB Chromogen: 40 µL DAB chromogen + 1 mL 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. To purchase separately from BioLegend, items are: AEC Chromogen **925804**, AEC substrate buffer **925903**; DAB Chromogen **926506**, DAB Substrate Buffer **926605**. If using a non-BioLegend chromogen, dilute and incubate according to the manufacturer's instructions.

15 Rinse slides with lab grade water.

16 Counterstain

1. Submerge slides in Hematoxylin for 30 seconds (not provided).
2. Rinse under running lab grade water for 1 minute or until water is clear.
3. Submerge slides in Bluing Reagent for 1 minute (not provided).
4. Rinse under running lab grade water for 1 minute.

17 Clear slides: Dehydrate the tissue.

1. 95% alcohol 3 minutes in (1) 250 mL container
2. 100% alcohol 5 minutes in each of (3) different 250 mL container
3. Xylene 5 minutes in each of (3) different 250 mL container

18 Coverslip

- Cover slip slide using Permanent Aqueous Mounting Medium or Xylene Based medium.

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