



SPECIFICATION SHEET

## Streptavidin Alkaline Phosphatase (Ready-To-Use)

### DESCRIPTION:

This reagent is intended to be used in a labeled streptavidin-biotin immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody which reacts with the primary antibody, enzyme-labeled streptavidin, and substrate-chromogen.

### AVAILABILITY:

<u>Catalog #</u>	<u>Volume</u>
TS-060-AP	60ml
TS-125-AP	125ml

### STAINING PROTOCOL:

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in buffer.
3. If required, incubate tissue in digestive enzyme (or appropriate pretreatment).
4. Wash 4 times in buffer.
5. (Optional) Place slide in Ultra V Block and incubate for 5-10 minutes at room temperature to block nonspecific background staining. Note: Do not exceed 10 minutes or there may be a reduction in desired stain.
6. Rinse (Optional).
7. Apply primary antibody and incubate according to manufacturer's protocol.
8. Wash 4 times in buffer.
9. Incubate slide with biotinylated secondary solution according to manufacturer's protocol.
10. Wash 4 times in buffer.
11. Incubate slide with **Streptavidin Alkaline Phosphatase** solution for 10 minutes at room temperature.
12. Rinse 4 times in buffer.
13. Place slide in appropriate chromogenic substrate and incubate until desired reaction is achieved.
14. Counterstain and coverslip.

47790 Westinghouse Dr. Fremont CA 94539 USA  
Tel: (800) 828-1628 Fax: (510) 991-2826  
Email: labvision@labvision.com Website: www.labvision.com



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Note: This reagent is provided in bulk form for use with automated systems or in staining jars for repeated use. If the DIP method is employed, staining jar should be sealed and refrigerated between uses. Remove staining jars from the refrigerator 30 minutes prior to staining to allow reagents to come to room temperature.

**STORAGE:** Store at 2-8°C.

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## TROUBLESHOOTING GUIDE

### OVERSTAINING:

1. Concentration of the primary antibody was too high or the incubation time was too long.
2. Temperature during incubation was too high.
3. Incubation time with link antibody or streptavidin/enzyme label was too long.

### NONSPECIFIC BACKGROUND STAINING:

1. Rinsing between steps was inadequate.
2. Tissue was allowed to dry with reagents on.
3. Folds in tissue trapped reagents.
4. Tissue contains endogenous enzyme.
5. Tissue contains endogenous biotin.
6. Antigen migrated in tissue.
7. Excessive tissue adhesive on slides.
8. Inadequate blocking with protein block.

### WEAK STAINING:

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1. Primary antibody concentration was too low or incubation time was too short.
2. Reagents are past their expiration date.
3. Reagent is reaching the end of its useful life.
4. Counterstain or mounting media were incompatible and dissolved the chromogen reaction product.
5. Room temperature was excessively cool.
6. The primary antibody does not recognize an antigen that survives fixation and embedding in high enough amounts.
7. Excessive incubation with protein block (Ultra Block or normal serum).

**NO STAINING:**

1. Steps were inadvertently left out.
2. There is no relevant antigen in the tissue.
3. The primary antibody is not compatible with secondary.
4. Chromogenic substrate does not match enzyme label.
5. One or more components have been inactivated.

**LIMITATIONS:**

This product is available for research use only; not for diagnostic or therapeutic work. Lab Vision Corporation will not be held responsible for patent infringement or other violation that may occur with the use of this product.