HistoMark®
Biotin Streptavidin-AP Systems

Catalog No.	Size
71-00-39	500 Slides
71-00-40	500 Slides
71-00-41	500 Slides

DESCRIPTION
HistoMark Biotin Streptavidin-Alkaline Phosphatase (AP) Systems provide rapid, precise localization of cell surface and intracellular antigens in frozen or paraffin-embedded tissue, cytospins and touch preparations. The kits contain normal goat serum, biotinylated secondary antibody and streptavidin labeled with alkaline phosphatase. The pre-diluted, liquid reagents are provided in convenient, controlled tip dropper bottles.

These products are designed for use with KPL's phosphatase HistoMark staining systems (See RELATED PRODUCTS) or other appropriate phosphatase substrate.

BACKGROUND
Streptavidin is a 60 kDa molecular weight protein isolated from *Streptomyces avidinii*[1,2]. Similar to egg white avidin, it displays a high affinity ($K_D=10^{-15}$) for biotin and has 4 binding sites for this low molecular weight vitamin. Streptavidin has an isoelectric point near 10. Avidin has a tendency toward non-specific binding when applied to negative-charged surfaces[3]. The lower isoelectric point of streptavidin greatly lessens this phenomenon.

Hsu et. al. devised a procedure using unlabeled primary antibody, a biotinylated secondary antibody, followed by addition of a pre-formed avidin-biotinylated peroxidase complex[4]. This is known as the ABC technique. Recently Shi et. al. suggested that the use of a biotinylated antibody followed by addition of streptavidin covalently coupled with alkaline phosphatase proved greater sensitivity than ABC methods[5]. This might be expected since technique variation with ABC procedures could result in saturation of all streptavidin (avidin) binding sites by biotinylated enzyme. Also, these results taken with the partial inactivation of AP by biotinylation[6], strongly suggest that use of AP conjugated to streptavidin is superior to a performed streptavidin-biotinylated AP complex. KPL uses carefully controlled technology to prepare AP-streptavidin and biotinylated secondary antibody conjugates. The incorporation of a long chain spacer into the biotinylated secondary antibody diminishes steric hindrance allowing rapid reaction with AP-streptavidin[7].

PRINCIPLE
Non-specific background staining is blocked using normal goat serum. After sections are reacted with an unlabeled primary antibody, a biotinylated secondary antibody is applied. Following incubation, the unreacted biotinylated antibody is removed by brief washing, and the sections are covered with a streptavidin-phosphatase conjugate. This reacts rapidly with the biotin attached to the secondary antibody. After washing, the streptavidin-phosphatase is visualized using one of KPL's HistoMark substrates.

KIT COMPONENTS
- SERUM BLOCK: Heat inactivated 10% v/v Normal Goat Serum with anti-microbial preservative added.

10% Normal Goat Serum	Catalog No. 71-00-27	50 mL

- BIOTINYLATED SECONDARY ANTIBODY: Supplied at a concentration of 2.0 µg/mL. Contains 100 mM Tris buffer, pH 7.6, stabilizers and proprietary preservative. One of the following:

Goat Anti-Mouse IgG	Catalog No. 71-00-29	50 mL
Goat Anti-Rabbit IgG	Catalog No. 71-00-30	50 mL
Goat Anti-Rat IgG	Catalog No. 71-00-31	50 mL

- PHOSPHATASE LABELED STREPTAVIDIN: Supplied at a concentration of 2.0 µg/mL. Contains 100 mM Tris buffer, pH 7.6, stabilizers and 0.05% sodium azide as preservative.

AP Streptavidin	Catalog No. 71-00-45	50 mL

STORAGE/STABILITY
Store at 2 - 8°C. Stable for a minimum of 1 year from date of receipt at 2 - 8°C. Discard solutions if microbial growth is evident.

REAGENTS REQUIRED, NOT PROVIDED
1. Primary antibody.
2. Wash buffers.
3. Phosphatase substrate (See RELATED PRODUCTS).
4. Reagent quality water (deionized, distilled water or equivalent).
5. Mounting media.

ACCESSORIES REQUIRED, NOT PROVIDED
Microscope, microscope slides, coverslips, pipettes, test tubes, humidity chamber.

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NOTES

1. DO NOT USE PHOSPHATE BUFFERED SALINE OR ANY BUFFER CONTAINING INORGANIC PHOSPHATE. INORGANIC PHOSPHATE IS AN INHIBITOR OF ALKALINE PHOSPHATASE.
2. Primary antibodies should be titrated to determine proper working dilutions.
3. Sections from mammalian intestine should not be used unless intestinal alkaline phosphatase is destroyed prior to application of Streptavidin-phosphatase.
4. Always incorporate a positive control, negative control and reagent control.
5. Do not use egg albumin to prevent sections from washing off slides. Traces of egg avidin may provide erroneous results. Instead use gelatin or poly-L-lysine.
6. Do not allow sections to dry out during incubations.
7. Remove as much buffer as possible after washes.
8. Water purified by reverse osmosis with a conductivity of 1 megohm or greater is recommended.
9. Low melting point paraffins (> 60°C) should be used to lessen antigen denaturation.
10. Fixation in freshly prepared 4% buffered paraformaldehyde will better preserve tissue antigens.

PROCEDURES

PARAFFIN SECTIONS

1. Rehydrate paraffin embedded sections through graded alcohol (3 minutes each in 100%, 80%, 40% and 20% ETOH) to water.
2. Soak slide for 5 minutes in a Coplin jar containing 100 mM Tris-HCl (See SOLUTION PREPARATION).
3. Proceed to step 1 of the General Procedure.

OTHER SPECIMENS (FROZEN SECTIONS, CYTOSPINS, ETC.)

1. Air dry slide-mounted sections for at least 1 hour.
2. Immediately before use, fix with a solution appropriate for the antigen to be detected. If sections are to be saved for an extended period, air dry for 1 hour after fixation. Wrap slides individually in aluminum foil and store desiccated at –70°C. Prior to use, remove from freezer and warm to room temperature for at least 1 hour before removing foil wrap.
3. Soak sample 10 - 15 minutes in a Coplin jar containing Tris-HCl.
4. Proceed to step 1 of the General Procedure.

GENERAL PROCEDURE

NOTE: If color develops too rapidly for your staining conditions, (i.e. less than one minute), further dilution of the primary antibody is recommended. An estimation of appropriate primary antibody dilution may be obtained by applying 1/50, 1/100, 1/200, 1/400 and 1/800 dilutions to tissue sections. The optimal dilution is the one that results in appropriate color development within 10 minutes without background staining.

APPLY SERUM BLOCK

1. Shake off buffer and wipe off excess buffer surrounding section.
2. Completely cover section with Normal Goat Serum.
3. Incubate 15 minutes at room temperature in a humidity chamber.
4. Soak in Tris-HCl for 5 minutes.

APPLY PRIMARY ANTIBODY

1. Shake off Tris and wipe off any excess surrounding section.
2. Completely cover section with diluted primary antibody.
3. Incubate 10 - 30 minutes at room temperature in a humidity chamber.
4. Rinse off primary antibody with Tris-HCl in a Coplin jar. Soak 5 minutes in same buffer.

APPLY BIOTINYLATED ANTIBODY

1. Shake off buffer and wipe off excess buffer surrounding section.
2. Completely cover section with biotinylated secondary antibody.
3. Incubate 10 - 30 minutes at room temperature in a humidity chamber.
4. Rinse off biotinylated antibody with Tris-HCl in a Coplin jar. Soak 5 minutes in same buffer.

APPLY STREPTAVIDIN PHOSPHATASE

1. Shake off buffer and wipe off excess buffer surrounding section.
2. Completely cover section with Streptavidin-Phosphatase.
3. Incubate 10 - 30 minutes at room temperature in a humidity chamber.
4. Rinse off Streptavidin-Phosphatase with Tris-HCl in a Coplin jar. Rinse 5 minutes in same buffer.

COLOR DEVELOPMENT

Develop color using one of KPL's HistoMark phosphatase substrates (See RELATED PRODUCTS) or other appropriate phosphatase substrate.

SOLUTION PREPARATION

- TRIS-HCL WORKING SOLUTION
  Dissolve 121 g of Tris Base in 500 mL reagent quality water. Adjust pH to 7.6 with approximately 200 - 300 mL 2M HCl. Q.S. to 1 Liter with reagent quality water to obtain a 100mM working buffer.
- TRIS BUFFERED SALINE WORKING SOLUTION
  Proceed as for Tris-HCl but add 70 g of NaCl prior to adjusting pH.

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TROUBLESHOOTING

Causes of Excess Staining:

1. Incomplete deparaffinization.
2. Excess tissue adhesive.
3. Use of egg albumin as a tissue adhesive.
4. Improper dilution of primary antibody.
5. Non-specific binding of proteins.

Causes of No Staining:

1. Neglecting to apply primary antibody, biotinylated antibody, streptavidin-phosphatase or any combination of the above.
2. Antigen destruction by processing procedures.
3. Improper fixation.
4. Allowing samples to dry completely during the procedure.
5. Failure to follow protocol.
6. Use of phosphate containing buffers.

Causes of Weak Staining:

1. Failure to remove most of the wash solution from section prior to adding immunologic reagents.
2. Improper primary antibody dilutions.
3. Allowing substrate solutions to stand for an excessive time before use.
4. Use of phosphate containing buffers.
5. Failure to follow protocol.

PRODUCT SAFETY AND HANDLING

This product is considered non-hazardous as defined by The Hazard Communication Standard (29 CFR 1910.1200). Avoid contact with skin and eyes. In case of contact or spillage, clean with copious amounts of water. Product may be disposed via a sanitary sewer.

REFERENCES

1. Chaiet, L.; Wolf, F.S. (1964). *Arch. Biochem. Biophys.* 106:1.
2. Stapley, E.O. et. al. (1963). *Antimicrob. Agents Chemotherap.* 3:20.
3. Woods, G.S.; Warnke, R. (1981). *J. Histochem. Cytochem.* 29:1196.
4. Hsu, H.M. et. al. (1981). *Am. J. Clin. Pathol.* 75:734.
5. Shi, Z. R.; Itzkowitz, S. H. (1988). *J. Histochem. Cytochem.* 36:317.
6. Guesdon, J.; Ternyck, T.; Avrameas, S. (1979). *J. Histochem. Cytochem*. 27:1131.
7. Leary, J.J.; Brigati, D.J.; Ward, D.C. (1983). *Proc. Natl. Acad. Sci.* (USA). 80: 4045.

RELATED PRODUCTS

HistoMark RED	Catalog No. 55-69-00
HistoMark BLUE	Catalog No. 55-70-00
Universal Block	Catalog No. 71-00-61

See KPL's catalog for a complete list of biotinylated antibodies, substrates, and complete kits for immunohistochemistry.

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