KPL HistoMark®
Biotin Streptavidin-HRP Systems

Catalog No.
5520-0023 (71-00-18)
5520-0024 (71-00-19)
5520-0025 (71-00-20)
5520-0026 (71-00-26)

DESCRIPTION

KPL HistoMark Biotin Streptavidin-HRP 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 or rabbit serum, biotinylated secondary antibody and streptavidin labeled with horseradish peroxidase.

Systems are available for use with:

Mouse primary antibody	5520-0023 (71-00-18)
Rabbit primary antibody	5520-0024 (71-00-19)
Rat primary antibody	5520-0025 (71-00-20)
Goat primary antibody	5520-0026 (71-00-26)

These products are designed for use with SeraCare's peroxidase HistoMark staining systems, or any peroxidase substrate.

KIT COMPONENTS

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

- KPL Normal Goat Serum (10%) 50 mL 5560-0007 (71-00-27)
- KPL Normal Rabbit Serum (10%) 50 mL 5560-0008 (71-00-28)

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

- KPL Goat Anti-Mouse 5570-0006 (71-00-29) 50 mL
- KPL Goat Anti-Rabbit 5570-0007 (71-00-30) 50 mL
- KPL Goat Anti-Rat 5570-0008 (71-00-31) 50 mL
- KPL Rabbit Anti-Goat 5570-0009 (71-00-37) 50 mL

PEROXIDASE LABELED STREPTAVIDIN: Supplied at a concentration of 2.0 µg/mL. Contains 100 mM Tris buffer, pH 7.6, stabilizers and preservative.

- HRP-Streptavidin 5550-0001 (71-00-38) 50 mL

FORM

The pre-diluted, liquid reagents are provided in convenient, controlled tip dropper bottles. Sufficient reagents are provided to process approximately 500 slides.

STORAGE/STABILITY

Store at 2–8°C. Stable for a minimum of 1 year from date of receipt at 2-8°C.

PRINCIPLE

Non-specific background staining is blocked using normal serum produced in the same animal that produced the secondary antibody. 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-peroxidase conjugate. This reacts rapidly with biotin attached to the secondary antibody. After washing, the streptavidin-peroxidase is visualized using one of SeraCare's HistoMark substrates.

REAGENTS REQUIRED, NOT PROVIDED

1. Primary antibody.
2. Wash buffers.
3. Peroxidase substrate (See RELATED PRODUCTS).
4. Reagent quality water (deionized, distilled water or equivalent).
5. Reagents for inhibiting endogenous peroxidase (See RELATED PRODUCTS).
6. Mounting media.

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

PROCEDURES
PARAFFIN SECTIONS

1. Deparaffinize slide-mounted sections in xylene or xylene substitutes through graded alcohols to water.
2. Rinse for 5 minutes in reagent quality water.
3. Rinse for 10 minutes in a buffer such as 100 mM Tris-HCl or Tris-buffered Saline (See SOLUTION PREPARATION).
4. Block endogenous peroxidase, if necessary, with SeraCare's KPLPeroxidase Blocking Solution (See RELATED PRODUCTS) or absolute methanol containing 0.3% H_2O_2 (See SOLUTION PREPARATION).
5. Proceed to step 1 of the General Procedure.

FROZEN SECTIONS

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.
3. Rinse 10 - 15 minutes in Tris-HCl buffer.
4. Block endogenous peroxidase, if necessary, with SeraCare's KPL4. Peroxidase Blocking Solution (See RELATED PRODUCTS) or 0.3% H_2O_2 in absolute methanol (See SOLUTION PREPARATION).
5. 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 KPL Normal Goat or KPL Rabbit Serum.
3. Incubate 15 minutes at room temperature in a humidity chamber.

APPLY PRIMARY ANTIBODY

1. Shake off serum and wipe off excess serum surrounding section.
2. Completely cover section with diluted primary antibody.
3. Incubate 30 minutes at room temperature in a humidity chamber.
4. Rinse off primary antibody with wash buffer. Rinse 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 30 minutes at room temperature.
4. Rinse off antibody with wash buffer. Rinse 5 minutes in same buffer.

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APPLY STREPTAVIDIN PEROXIDASE

1. Shake off buffer and wipe off excess buffer surrounding section.
2. Completely cover section with KPL Streptavidin-Peroxidase.
3. Incubate 30 minutes at room temperature.
4. Rinse off KPL Streptavidin-Peroxidase with wash buffer. Rinse 5 minutes in same buffer.

COLOR DEVELOPMENT

Develop color using one of SeraCare's HistoMark peroxidase substrates (See RELATED PRODUCTS) or other appropriate peroxidase 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 mL2M 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.

0.3% H_2O_2 IN ABSOLUTE METHANOL[8]

Prepare 0.3% H_2O_2 in 100% MeOH. Incubate slides for 30 minutes H_2O_2/methanol solution. Rinse 10 - 15 minutes in Tris-HCl buffer.

TROUBLESHOOTING

Causes of Excess Staining:

1. Failure to block endogenous peroxidase.
2. Incomplete deparaffinization.
3. Excess tissue adhesive.
4. Improper dilution of primary antibody
5. Non-specific binding of proteins.

Causes of No Staining:

1. Neglecting to apply either primary antibody, biotinylated antibody, streptavidin-peroxidase or any combination of the above.
2. Antigen destruction by processing procedures.
3. Improper fixation.
4. Use of azide in wash solution.
5. Allowing samples to dry completely during the procedure.
6. Failure to follow protocol.

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 solution to stand for an excessive time before use.
4. Deterioration of H_2O_2 solutions.

NOTES

1. The use of sodium azide is not recommended when working with horseradish peroxidase.
2. The use of hypochlorite-containing detergents for cleaning should be avoided.
3. Always incorporate a positive control, negative control and reagent control.
4. 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.
5. Do not allow sections to dry out during incubations.
6. Remove as much buffer as possible after washes.
7. Water purified by reverse osmosis with a conductivity of 1 megaohm or greater is usually of sufficient purity for immunoperoxidase techniques.
8. Low melting point paraffins (> 60°C) should be used to lessen antigen denaturation.
9. Fixation in freshly prepared 4% buffered paraformaldehyde will better preserve tissue antigens.

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BACKGROUND

Streptavidin is a 60 kd molecular weight protein isolated from *Streptomyces avidinii*[1,2]. Similar to egg white avidin, it displays a high affinity ($K_D=10^{-15}$)[1] 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 nonspecific 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. Then Shi et al suggested that the use of a biotinylated antibody followed by addition of streptavidin covalently coupled with horseradish peroxidase 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, the proposed crosslinking of avidin with biotinylated peroxidase, forming a high molecular weight complex, could sterically hinder reaction with biotinylated secondary antibody.

Controlled conjugation of horseradish peroxidase with streptavidin produces a product of lower molecular size, fully reactable with biotin attached to the secondary antibody. SeraCare conjugates biotin to antibodies via a long carbon spacer arm, further reducing the possibility of steric hindrance when reacted with enzyme-labeled streptavidin[6,7]. The increased sensitivity may allow greater dilution of primary antibodies (2 - 10 fold).

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. Leary, J.; Brigati, D.; Ward, D. (1983). *PSOC Nat'l. Acad. Sci. USA.* 80:4045.
7. Kendall, C. et. al. (1983). *J. Immunol. Methods*. 56:329.
8. Argenyi, Z. et. al. (1988). *Am. J. Clin. Pathol.* 90:622.

RELATED PRODUCTS	CAT. NO.
KPL DAB Reagent Set	5510-0031 (54-10-00)
KPL *Stable*DAB® Peroxidase Substrate	5510-0032 (54-11-00)
KPL TrueBlue® Peroxidase Substrate	5510-0030 (50-78-02)
KPL HistoMark® ORANGE	5510-0033 (54-74-00)
KPL HistoMark® BLACK	5510-0034 (54-75-00)
KPL Blocking Solution	5560-0006 (71-00-10)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.