

KPL HistoMark® BLACK

for Localization of Horseradish Peroxidase-Labeled Reagents

Catalog No.

5510-0034 (54-75-00)

Size

500 mL

DESCRIPTION

The KPL HistoMark BLACK Substrate System is an enhanced metal-bridging DAB procedure for visualization of horseradish peroxidase (HRP)-labeled reporter reagents. The substrate system provides a brown-black specific stain with green counterstain for immunohistochemical staining. This procedure employs cobalt-based DAB enhancement, providing improved contrast compared to standard DAB procedures.

KIT COMPONENTS

KIT COMPONENTS	CAT. NO.
KPL DAB Solution	5510-0041 (71-00-08)
KPL Peroxide Solution	5570-0004 (71-00-09)
KPL Blocking Solution	5560-0006 (71-00-10)
KPL Contrast Green	5540-0003 (71-00-11)
KPL Enhance Black	5510-0042 (71-00-12)

Sufficient reagents are supplied to prepare 500 mL of substrate solution (approximately 1000 slides).

STORAGE/STABILITY

Reagents are stable for a minimum of one year stored at 2 - 8°C. Discard solutions that become turbid. Warm all reagents to room temperature (24 - 28°C) before use.

REAGENTS NOT INCLUDED

1. Primary antibody.
2. HRP labeled reagents. KPL HistoMark Biotin Streptavidin Systems provide biotin-labeled secondary antibody, HRP labeled Streptavidin and Serum Block.
3. Isopropyl alcohol.
4. Mounting media (aqueous or xylene-based).
5. 0.1M TRIS-HCl or PBS (see BUFFER PREPARATION).

PREPARATION

- Substrate Solution (prepare immediately before use in Step 10):
 - a. Add 0.5 mL KPL Enhance BLACK Buffer Solution to 5 mL reagent quality water.
 - b. Add 0.1 mL of KPL DAB-C Solution.
 - c. Add 0.1 mL KPL Peroxide Solution.
 - d. Mix thoroughly. Use solution immediately.
- KPL Contrast GREEN Solution: supplied at use dilution.
- KPL Blocking Solution: Dilute 1/10 with reagent quality water (i.e. 1 mL of Blocking Solution

Concentrate to 9 mL reagent quality water). Diluted solution may be stored tightly capped at 2–8°C for up to one week.

1. Rehydrate paraffin embedded sections through graded alcohol (3 minutes each in 100%, 80%, 40% and 20% EtOH) to water. Other samples listed below do not need or require rehydration. Frozen sections must be thoroughly dried before use.
2. To block endogenous peroxidase activity, immerse samples in diluted KPL Blocking Solution as follows:
 - a. Frozen sections 45 seconds
 - b. Paraffin sections 4 minutes
 - c. Cytospin preparations 45 seconds
 - d. Blood films 45 seconds
 - e. Touch or squash preparations 1 minute.
 - f. Floating or whole sections 5 minutes
3. Rinse 5 minutes in reagent quality water.
4. Soak in 0.1M Tris-HCl or PBS 10 minutes.
5. Treat sample with primary antibody diluted in Tris-HCl or PBS 15 - 20 minutes.
NOTE: Extended incubation may improve sensitivity.
6. Wash sample with 0.1M Tris-HCl or PBS 10 minutes.
7. Incubate sample with biotin-labeled antibody directed against the primary antibody host species 15 - 20 minutes. If using HRP-labeled secondary antibody, proceed to Step 9.
8. Wash as in Step 6.
9. Shake off excess buffer and incubate sample with HRP Streptavidin or HRP labeled secondary antibody diluted in Tris-HCl or PBS 15 - 20 minutes.
10. Wash as in Step 6. Prepare Substrate Solution during this step.
11. Shake off excess buffer and cover section with Substrate Solution.
12. Incubate 10 minutes at room temperature out of direct light.
13. Rinse slide 2 - 3 minutes in reagent quality water.
14. Counterstain in Contrast GREEN Solution: paraffin embedded and frozen sections for 3 minutes; touch preparations, cytospin preparations and blood films for 30 - 45 seconds.

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15. Rinse thoroughly in 2 - 3 changes of isopropyl alcohol or until excess stain is removed from slide. DO NOT USE WATER OR ETHANOL SOLUTIONS.
16. Air dry and mount in aqueous or xylene-based mounting medium.

- d. Dilute 1 part stock from step 5c with 9 parts reagent quality water and mix well.

Phosphate Buffered Saline (PBS)

- a. Add PBS (0.01M), 8.0 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, 0.24 g KH₂PO₄.
- b. Adjust pH to 7.4 with 2M HCl.
- c. QS to 1 L with reagent quality.

RESULTS

- Sites of enzyme activity range from dark brown to black. Nuclei appear a contrasting pale green.
- Sections not reacted with primary antibody as a negative control should not develop a black tint.
- Further dilution of primary antibody or HRP-labeled reagent may be required to prevent excessive background.

NOTES

1. Always incorporate appropriate positive and negative controls.
2. The following method of disposal is recommended for solutions containing DAB:
 - a. Add 100 mL of household bleach to 2 Liters of water. Pour this solution into a 1 gallon plastic bottle.
 - b. Pour waste DAB solution into the solution from step 2a and mix by shaking. No more than 500 mL of DAB solution should be added. After last addition, allow container to stand at least 24 hours before discarding.
3. Instant development of a black color indicates that the primary antibody or peroxidase labeled reagent must be further diluted.
4. Prolonged incubation in substrate may increase background and inhibit nuclear counterstaining.
5. As an alternative method to block endogenous peroxidase, incubate slides for 30 minutes in 0.3% (w/v) H₂O₂ in absolute methanol followed by a 10 - 15 minute rinse in 0.1M Tris-HCl, pH 7.6 or similar buffer.

BUFFER PREPARATION

0.1M TRIS-HCl

- a. Dissolve 121 g Tris in 500 mL reagent quality water.
- b. Adjust pH to 7.6 with 2M HCl (approximately 300 mL).
- c. QS to 1 L with reagent quality water to obtain a 1M stock.

PRINCIPLE

The application of antibodies and other proteins covalently coupled to horseradish peroxidase (HRP) in immunohistology is well documented⁽¹⁻⁴⁾. It is the most frequently used label for immunohistologic techniques. In the presence of peroxide (H₂O₂), HRP catalyzes the oxidation of phenols, naphthols, diamines, aminophenols, indophenols etc. forming chromogenic products visible by light microscopy. Most commonly employed are 3-amino-9-ethylcarbazole⁽⁵⁾, pphenylenediamine/ catechol⁽⁶⁾, 4-Chloro-1-Naphthol⁽⁷⁾ and Diaminobenzidine (DAB)⁽⁸⁾. Although a suspected carcinogen, DAB is the most widely accepted donor substrate for peroxidase immunohistochemistry, since it provides a reaction product insoluble in alcohols and xylene. The DAB oxidation product is light brown and may be difficult to visualize if low antigen density occurs. The KPL HistoMark BLACK system utilizes metal bridging techniques for improved staining intensity^(3,9). Endogenous peroxidase activity is eliminated by controlled oxidation prior to application of antibodies⁽¹⁰⁾.

REFERENCES

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