KPL -diaminobenzidine) Substrate Solution

Catalog No.	Size
5510-0041 (71-00-08)	10 mL

DESCRIPTION

KPL DAB (3,3'-diaminobenzidine) Substrate Solution deposits a brown specific stain in the presence of horseradish peroxidase (HRP). DAB is useful for immunohistochemical and immunoblotting applications.

CONTENTS

KPL DAB Substrate Solution contains 3,3'-diaminobenzidine at 25 mg/mL. Sufficient material is supplied to prepare 500 mL Substrate Solution.

Caution: DAB is a suspected carcinogen. Avoid contact with skin or clothing. Follow recommended disposal procedures.

FORM/STORAGE/STABILITY

KPL DAB Substrate Solution is a 10 mL stable liquid concentrate. Store refrigerated at 2-8°C. Stable for a minimum of one year stored at 2-8°C. Warm to room temperature (24-28°C) before use.

REAGENTS NOT INCLUDED

1. Primary antibody.
2. HRP-labeled reagents
3. KPL Hydrogen peroxide (H_2O_2) solution
4. Isopropyl alcohol.
5. Mounting media (aqueous or xylene-based).
6. KPL Contrast BLUE Solution.
7. 0.1 M Tris-HCl buffer/wash solution, pH 7.6:
   a. Dissolve 121 g Tris in 500 mL reagent quality water.
   b. Adjust pH to 7.6 with 2 mol/L HCl (approximately 300 mL).
   c. QS to 1 L with reagent quality water to obtain a 1 M stock.
   d. Dilute 1 part stock from step 5c with 9 parts reagent quality water and mix well.

PREPARATION

a. Add 0.1 mL DAB Solution to 5 mL Tris-HCl buffer.
b. Add 0.1 mL Peroxide Solution
c. Mix thoroughly.
d. Use solution immediately.

PROCEDURE

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 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 0.1 M 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 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 SubstrateSolution.
12. Incubate 10 minutes at room temperature out of direct light.
13. Rinse slide 2 - 3 minutes in reagent quality water.
14. Counterstain with KPL Contrast BLUE if desired. Paraffin-embedded and frozen sections for 3 minutes; touch preparations, cytospin preparations and blood films for30 - 45 seconds.
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 mountingmedium.

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RESULTS

- Sites of enzyme activity range from light to dark brown. If counterstained, nuclei appear a contrasting blue.
- Sections not reacted with primary antibody as a negative control should not develop a brown 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 brown 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) H_2O_2 in absolute methanol followed by a 10 - 15 minute rinse in 0.1 mol/L Tris-HCl, pH 7.6 or similar buffer.
6. KPL DAB Reagent Set provides DAB, H_2O_2 and Tris solutions at use dilution in convenient dropper bottles.
7. SeraCare provides KPL HistoMark® BLACK and KPL HistoMark® ORANGE Substrate Systems which utilize metal bridging techniques for improved staining intensity and contrast compared to standard DAB techniques.

PRINCIPLE

The application of antibodies and other proteins covalently coupled to horseradish peroxidase (HRP) in immunohistology is well documented [4]. It is the most frequently used label for immunohistologic techniques. In the presence of peroxide (H_2O_2), HRP catalyzes the oxidation of phenols, naphthols, diamines, minophenols, indophenols, etc. forming chromogenic products visible by light microscopy. Most commonly employed are 3-amino-9-ethylcarbazole [5], pphenylenediamine/catechol [6], 4-chloro-l-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 oxidation of DAB results in formation of a free radical intermediate which polymerizes to form a brown product. DAB may be employed for demonstration of endogenous peroxidase and catalase activity; cytochrome oxidase; cupric ferrocyanide; and hemoproteins such as hemoglobin, myoglobin, and cytochrome c. Treatment of the DAB product with osmium, silver, cobalt or nickel will intensify final color. Reaction with osmium tetraoxide results in an electron opaque osmium black useful for ultrastructure research.

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REFERENCES

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