DAB Reagent Set

Catalog No.	Size
54-10-00	500 Slides

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

CONTENTS
The DAB Reagent Set provides a three component liquid substrate system in convenient dropper bottles.
15 ml Tris Buffer (0.1 M) Cat. No. 71-00-47
10 ml DAB Solution (25 mg/ml) Cat. No. 71-00-46
10 ml Peroxide Solution (0.5%) Cat. No. 71-00-48
Sufficient reagents are supplied to stain approximately 500 slides.

STORAGE/STABILITY
Store reagents at 2-8°C. Stable for a minimum of one year from date of receipt when stored at 2-8°C.

SUGGESTED REAGENTS NOT INCLUDED
1. Primary antibody.
2. Peroxidase blocking solution (See RELATED PRODUCTS) or H_2O_2.
3. HRP-labeled secondary antibody or streptavidin (See RELATED PRODUCTS).
4. Contrast BLUE (See RELATED PRODUCTS) or hematoxylin.
5. Isopropyl alcohol.
6. Mounting media.
7. 0.1 M Tris-HCl or PBS (See SOLUTION PREPARATION).

PREPARATION
Note: Warm reagents to room temperature before use.
1. Add 3 drops (≈150 µl) Tris Buffer Concentrate to 5 ml reagent quality water.
2. Add 2 drops (≈100 µl) DAB Solution.
3. Add 2 drops (≈100 µl) Peroxide Solution.

Mix solution thoroughly and use immediately. The working substrate solution contains 0.5 mg/ml DAB and 0.1% H_2O_2.

STAINING 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. Block endogenous peroxidase activity by immersing samples in KPL's Peroxidase Blocking Solution as follows. (If using H_2O_2 see TROUBLESHOOTING.)
    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 five 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 biotinylated antibody, directed against the primary antibody host species, 15-20 minutes. If using HRP-labeled secondary antibody, go 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 DAB substrate during this step.)
11. Shake off excess buffer and cover section with DAB substrate.
12. Incubate 10 minutes at room temperature out of direct light.
13. Rinse slide 2-3 minutes in reagent quality water.
14. Counterstain with Contrast BLUE, or hematoxylin, if desired:
    a. Paraffin embedded and frozen sections for 3 minutes
    b. Touch preparations, cytospin preparations and blood films for 30-45 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 with aqueous or xylene-based mounting medium.

Note: KPL recommends TrueBlue™ Peroxidase Substrate (See RELATED PRODUCTS) for double labeling.

RESULTS
1. Sites of enzyme activity range from light to dark brown.
2. If counterstained, nuclei appear a contrasting blue.

3. Sections not reacted with primary antibody as a negative control should not develop a brown tint.
4. To prevent background, further dilution of primary antibody or HRP-labeled reagent may be required.

TROUBLESHOOTING

1. Always incorporate appropriate positive and negative controls.
2. Instant development of brown color indicates that the primary antibody or peroxidase-labeled reagent must be further diluted.
3. Prolonged incubation in substrate may increase background and inhibit nuclear counterstaining.
4. 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 M Tris-HCI, pH 7.6 or PBS.

DISPOSAL

The following method of disposal is recommended for solutions containing DAB:

1. Add 100 ml of household bleach to 2 liters of water. Pour solution into a 1 gallon plastic bottle.
2. Pour waste DAB solution into the bleach solution and mix by shaking. No more then 500 ml of DAB solution should be added.
3. After last addition, allow container to stand at least 24 hours before discarding.

BUFFER PREPARATION

0.1 M TRIS-HCl

1. Dissolve 121 g Tris in 500 ml reagent quality water.
2. Adjust pH to 7.6 with 2 M HCl (approximately 300 ml).
3. QS to 1 L with reagent quality water to obtain a 1 M stock.
4. Dilute 1 part stock from step 5c with 9 parts reagent quality water and mix well.

Phosphate Buffered Saline (PBS)

1. Add PBS (0.01 M), 8.0 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4.
2. Adjust pH to 7.4 with 2 M HCl.
3. QS to 1 L with reagent quality water and mix well.

REFERENCES

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PRODUCT SAFETY AND HANDLING

This product is considered hazardous as defined by the Hazard Communication Standard (29 CFR 1910.1200). Avoid contact with skin and eyes. In case of contact or spillage, consult MSDS. Dispose of this product as instructed above.

RELATED PRODUCTS

Peroxidase Blocking Solution	Cat. No. 71-00-10
Contrast BLUE	Cat. No. 71-00-06
TrueBlue® Peroxidase Substrate	Cat. No. 71-00-64
HistoMark® Biotin/Streptavidin Kits for use with:	
• Mouse Primary Antibody	Cat. No. 71-00-18
• Rabbit Primary Antibody	Cat. No. 71-00-19
• Rat Primary Antibody	Cat. No. 71-00-20
• Goat Primary Antibody	Cat. No. 71-00-26

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, 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), p-phenylenediamine/catechol (6), 4-chlorol-napthol (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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