ReliableDAB Peroxidase Substrate Kit

Catalog Number KA0562

500 slides

Version: 02

Intended for research use only

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Table of Contents

Introduction

Intended Use

Stable DAB (3,3'-diaminobenzidine) deposits a brown specific stain in the presence of horseradish peroxidase (HRP)-labeled reporter reagents. The convenient 2-Component system is stable after mixing for 1 week at room temperature or 2 weeks when refrigerated. This reduces waste and minimizes exposure to hazardous substances. The substrate is useful for immunohistochemical or immunoblotting applications.

Principle of the Assay

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.

General Information

Materials Supplied

List of component

Component	Amount
Stable DAB Solution	2 mL
Buffer Solution	100 mL

Storage Instruction

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

- ✓ DAB solution should appear light to medium brown. Discard solution if it appears dark purple or if heavy precipitate develops.
- ✓ After mixing, Stable DAB substrate is stable for 7 days at room temperature or 14 days at 2 - 8°C.

Materials Required but Not Supplied

- ✓ Primary antibody.
- ✓ Peroxidase blocking solution or H_2O_2.
- ✓ HRP-labeled secondary antibody or streptavidin.
- ✓ Contrast BLUE or hematoxylin.
- ✓ Isopropyl alcohol.
- ✓ Mounting media.
- ✓ 0.1 M Tris-HCl or PBS (See Reagent Preparation).

Precautions for Use

The product listed herein is for research use only. Nothing disclosed herein is to be construed as a recommendation to use this product in violation of any patents. The information presented above is believed to be accurate. However, said information and product are offered without warranty or guarantee since the ultimate conditions of use and the variability of the materials treated are beyond our control. We cannot be responsible for patent infringements or other violations that may occur with the use of this product. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

Assay Protocol

Reagent Preparation

Note: Warm reagents to room temperature before use.

✓ Substrate solution

- Add 2 drops of Stable DAB Solution to 5 mL Buffer Solution in an opaque sealable container.
- Mix solution thoroughly.
- After use, cap and store at room temperature for up to 7 days or 2 - 8°C for up to 14 days. Discard after maximum storage time.

✓ 0.1 M Tris-HCl:

- Dissolve 121 g Tris in 500 mL reagent quality water.
- Adjust pH to 7.6 with 2 M HCl (approximately 300 mL).
- QS to 1 Liter with reagent quality water to obtain 1M stock solution.
- Dilute 1 part stock from Step 5 with 9 parts reagent quality water and mix well.

✓ Phosphate Buffered Saline (PBS):

- 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.
- Adjust pH to 7.4 with 2 M HCl.
- QS to 1 Liter with reagent quality water and mix well.

Assay Procedure

1. Place slides in a Xylene bath and incubate for 5 minutes. Change baths and repeat once.
2. 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.
3. Block endogenous peroxidase activity by immersing samples in Abnova's Peroxidase Blocking Solution as follows. (If using H_2O_2 see Troubleshooting.)
   - ✓ Frozen sections 45 seconds
   - ✓ Paraffin sections 4 minutes
   - ✓ Cytospin preparations 45 seconds
   - ✓ Blood films 45 seconds
   - ✓ Touch or squash preparations 1 minute
   - ✓ Floating or whole sections 5 minutes
4. Rinse five minutes in reagent quality water.
5. Soak in 0.1 M Tris-HCl or PBS 10 minutes.
6. Treat sample with primary antibody diluted in Tris-HCl or PBS 15 - 20 minutes.

Note: Extended incubation may improve sensitivity.

7. Wash sample with Tris-HCl or PBS 10 minutes.
8. 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.
9. Wash as in Step 6.
10. Shake off excess buffer and incubate sample with HRP Streptavidin or HRP-labeled secondary antibody diluted in Tris-HCl or PBS, 15 - 20 minutes.
11. Wash as in Step 6. (Prepare Stable DAB substrate during this step.)
12. Shake off excess buffer and cover section with Stable DAB substrate.
13. Incubate 5 - 10 minutes or until brown color is evident at room temperature and out of direct light.
14. Rinse slide 2 - 3 minutes in reagent quality water.
15. Counterstain with Contrast BLUE or hematoxylin, if desired:
    - ✓ Paraffin embedded and frozen sections for 3 minutes.
    - ✓ Touch preparations, cytospin preparations and blood films for 30 - 45 seconds.
16. Rinse thoroughly in 2 - 3 changes of isopropyl alcohol or until excess stain is removed from slide. DO NOT USE WATER OR ETHANOL SOLUTIONS.
17. Air dry and mount with aqueous or Xylene-based mounting medium.

✓ 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.

Data Analysis

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.

Resources

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.

References

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