KPL *Stable*DAB® Peroxidase Substrate

Catalog No.	Size
5510-0032 (54-11-00)	100 mL

DESCRIPTION

KPL *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.

CONTENTS

KPL *Stable*DAB consists of two liquid components:

- 1 x 2 mL KPL *Stable*DAB Solution
- 1 x 100 mL KPL Buffer Solution

5510-0032 (54-11-00) contains sufficient 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.

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

SUGGESTED REAGENTS NOT INCLUDED

1. Primary antibody.
2. KPL Peroxidase Blocking Solution (See RELATED PRODUCTS) or H_2O_2.
3. HRP-labeled secondary antibody or streptavidin (See RELATED PRODUCTS).
4. KPL 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 2 drops KPL *Stable*DAB Solution to 5 mL KPL Buffer Solution in an opaque sealable container.
2. Mix solution thoroughly.
3. 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.

STAINING 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 KPL 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
4. Rinse five minutes in reagent quality water.
5. Soak in 0.1 M Tris-HCl or PBS 10 minutes.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. If using an HRP-labeled secondary antibody, go to Step 9.
7. If using a biotinylated antibody, incubate sample with biotinylated antibody that is directed against the primary antibody host species for 15 - 20 minutes.
8. Wash sample as instructed in Step 6.
9. Shake off excess buffer and incubate sample with KPL HRP Streptavidin or KPL HRP-labeled secondary antibody diluted in Tris-HCl or PBS, 15 - 20 minutes.
10. Wash as in Step 6. (Prepare KPL *Stable*DAB substrate during this step.)

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11. Shake off excess buffer and cover section with KPL *Stable*DAB substrate.
12. Incubate 5 - 10 minutes or until brown color is evident at room temperature and out of direct light.
13. Rinse slide 2 - 3 minutes in reagent quality water.
14. Counterstain with KPL 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: SeraCare recommends KPL 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-HCl, 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.
2. Pour solution into a 1 gallon plastic bottle.
3. Pour waste DAB solution into the bleach solution and mix by shaking. No more than 500 mL of DAB solution should be added.
4. 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,
2. 0.24 g KH_2PO_4.
3. Adjust pH to 7.4 with 2 M HCl.
4. QS to 1 L with reagent quality water and mix well.

RELATED PRODUCTS	CAT. NO.
KPL Peroxidase Blocking Solution	5560-0006 (71-00-10)
KPL Contrast BLUE	5540-0002 (71-00-06)
KPL TrueBlue® Peroxidase Substrate	5510-0049 (71-00-64)
HistoMark® Biotin/Streptavidin Kit - Mouse Primary Antibody	5520-0023 (71-00-18)
HistoMark® Biotin/Streptavidin Kit - Rabbit Primary Antibody	5520-0024 (71-00-19)
HistoMark® Biotin/Streptavidin Kit - Rat Primary Antibody	5520-0025 (71-00-20)
HistoMark® Biotin/Streptavidin Kit - Goat Primary Antibody	5520-0026 (71-00-26)

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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], ρ-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.

PRODUCT SAFETY AND HANDLING

See SDS (Safety Data Sheet) for this product.

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

REFERENCES

1. Nakane, P.K. and Pierce, G.B. Jr. (1966). *J. Histochem. Cytochem.* 14: 929.
2. Sternberger, L.A. et. al. (1970). *J. Histochem. Cytochem.* 18:315.
3. Hsu, S.M. and Ree, H.J. (1980). *Am. J. Clin. Pathol.* 74: 32.
4. DeJong, A. et. al. (1985). *Histochem.* 17: 1119.
5. Graham, R. et. al. (1965). *J. Histochem. Cytochem.* 13: 150.
6. Hanker, J.S. et. al. (1977). *Histochem.* 9: 789.
7. Nakane, P.K. (1968). *J. Histochem. Cytochem.* 16: 557.
8. Graham, R.C. Jr. and Karnovsky, M.J. (1966). *J. Histochem. Cytochem.* 14: 291.