

TECHNICAL DATA SHEET 164

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3, 3'-Diaminobenzidine Tetrahydrochloride

(DAB•4HCl) Immunochemical Grade in Prewieghed Serum Vials

Soluble and Safe to Handle:

The DAB•4HCl is supplied, ready to use, in preweighed serum vials which contain 10 mg or 25 mg of soluble material. Contact with the solid DAB•4HCl is avoided by adding buffer directly into the vial with a hypodermic syringe. Our DAB•4HCl is prepared to the highest purity standards and is used without additional purification. Storage at room temperature for short periods of time is acceptable. For long-term storage, 0°C is recommended.

Preparing DAB Solution:

Dissolve to a concentration of 0.5mg per ml in buffer. Use immediately or store aliquots for future use at 0°C - 20°C. Do not freeze entire amount defrost and refreeze. It will cause precipitation of the DAB.

History and Applications:

Graham and Karnovsky¹ introduced 3,3'-diaminobenzidine tetrahydrochloride (DAB) for the ultrastructural demonstration of the tracer protein, horseradish peroxidase (HRP). Later Seligman, et al.,² demonstrated ultrastructurally, the terminal cytochrome oxidase of the mitochondrial electron transport chain with DAB•4HCl via cytochrome C. Novikoff, et al.,³ Fahimi,⁴ and Vigil⁵ have demonstrated peroxisomes by virtue of the peroxidatic activity of their catalase. Phi bodies may be detected in leukocytes of patients with acute non-lymphocytic leukemia with DAB•4HCl.⁶ It is widely employed to label cells or origin of CNS pathways by staining HRP transported in axons. DAB•4HCl has also been useful in studying the tissue distribution of endogenous peroxidase⁷ and myoglobin⁸ activities as well as various exogenous tracer proteins: peroxidases of differing molecular weights and dimensions, hemoproteins⁹ including cytochrome C¹⁰ and myoglobin¹¹. It has been used for showing photo-oxidation in chloroplast lamellae¹² and also for *in situ* studies on the function and development of membrane systems that occur in anoxygenic photosynthetic bacteria during growth under different conditions¹³. Nucleic acid staining,¹⁴ staining specific for hemoglobin in polyacrylamide gels,¹⁵ and quantitation of catalase activity,¹⁶ are a few other applications employing DAB•4HCl.

Hanker, et al.,¹⁷ have shown that small amounts of cupric ferrocyanide (Hatchett's brown) localized as a primary reaction product at the subcellular sites of many enzymes may be amplified by subsequent stepwise treatment with DAB•4HCl and OsO₄. This is due to the ability of cupric ferrocyanide to oxidize DAB•4HCl catalytically. This technique has improved the demonstration of cholinesterases,¹⁸ non-specific esterase and acid phosphatase,¹⁹ monoamine oxidase, and mitochondrial dehydrogenases.²⁰

When HRP is used as a marker for the detection of antigens or antibodies by immunoperoxidase techniques, a high sensitivity of the cytochemical method for peroxidase is often required. Straus²¹ has shown that imidazole increases the sensitivity of the cytochemical reaction for peroxidase with DAB•4HCl at a neutral pH. Color modification of this reaction by metallic ions has been applied for double immunohistochemistry,²² while metal compounds, such as gold chloride, have been used to intensify the electron density of the DAB•4HCl reaction product.²³ An interference filter combination has recently been developed for contrast enhancement of the brownish HRP-activated DAB•4HCl reaction product in black and white photomicrography.²⁴ All of the reactions mentioned above are based on the rapid oxidation and polymerization of DAB•4HCl resulting in the formation of amorphous osmiophilic polymers, which are insoluble in water or lipid. The indamine-type polymers obtained on osmication are insoluble in plastic embedding media, have high electron opacity, and delineate structure reliably.

Because of the sharp histochemical localization of peroxidase with DAB•4HCl and because of the purity of peroxidase and structural considerations, this enzyme is the preferred tracer in modern immunocytochemistry. It is linked to tissue antigen by immunologic bonds and revealed with hydrogen peroxide as substrate and DAB•4HCl as electron donor.²⁵

Precautions:

DAB•4HCl, if swallowed, inhaled, or absorbed through the skin, may be harmful. Causes severe skin and eye irritation. Avoid breathing vapors or mist. Wash thoroughly after

handling. Use with adequate ventilation in a hood. Wear protective goggles, gloves and clothing. In case of contact with eyes, immediately flush with plenty of water and get medical attention. Diaminoben-zidine appeared to be inactive in a carcinogenicity screening test using rats,²⁶ but some individuals claim that it is a carcinogen.

Disposal:

To clean up small spills or leakage, cover with powdered limestone and sweep up. Waste can be disposed in a landfill or incinerated in accordance with applicable local, state, or federal regulations. Material can also be treated by dissolving in copious quantities of household bleach, and after letting it stand overnight, it should be flushed down the drain with plenty of water.

Ordering Information:

Cat. #	Description	Size
04001-2	DAB-4HCl, Immunochemical Grade	2x25mg in 60ml vials
04001-5	DAB-4HCl, Immunochemical Grade	5x25mg in 60ml vials
04008-5	DAB-4HCl, Immunochemical Grade	5x10mg in 30ml vials

Peroxide Antiperoxidase (PAP), Lyophilized

Cat. #	Description	Size
06382	Rabbit,	1ml
16815	Rat,	0.25ml
16709	Sheep,	1ml

Other Chromogens

Cat. #	Description	Size
08661	Hanker-Yates Reagent,	5 x 1g amp

To Order:

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In The U.S. FAX: 1-800-343-3291 • 215-343-0214

Order online anytime at www.polysciences.com

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