

Technical Data Sheets

3,3'-Diaminobenzidine Tetrahydrochloride (DAB 4HCl)

EMS Catalog #13080

Soluble and Safe to Handle:

By adding buffer directly into the vial with a hypodermic syringe, contact with the solid DAB.4HCl is avoided. Our DAB.4HCl is prepared to the highest purity standards and is used without further purification.

History and Applications:

Graham and Karnovsky introduced 3,3'diaminobenzidine tetrahydrochloride for the ultrastructural demonstration of the trace protein horseradish peroxidase (HRP). Later Seligman, et al., demonstrated ultrastructurally the terminal cytochrome oxidase of the mitochondrial electron transport chain with DAB.4HCl via cytochrome C. Novikoff, et al., Fahimi and Vigil have demonstrated peroxisomes by virtue of the peroxidatic activity of their catalase. Ph bodies may be detected in leukocytes of patients with acute non-lymphocytic leukemia with DAB.4HCl. 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 peroxidase and myoglobin activities as well as various exogenous tracer proteins: peroxidases of differing molecular weights and dimensions, heme proteins including cytochrome C, and myoglobin. It has been used for showing photo-oxidation in chloroplast lamellae and also for in situ studies on the function and development of membrane systems that occur in anoxygenic photosynthetic bacteria during growth under different conditions. Nucleic acid staining, staining specific for hemoglobin in polyacrylamide gels and quantitation of catalase activity are a few other applications employing DAB.4HCl.

Hanker, et al., have shown that small amounts of Cupric Ferrocyanide (Hatchett's brown) localized as a primary reaction product as the subcellar 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 catalytically oxidize DAB.4HCl and has improved the demonstration of cholinesterases, nonspecific esterase and acid phosphatase, monoamine oxidase and mitochondrial dehydrogenases.

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. Straus 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, while metal compounds such as gold chloride have been used to intensify the electron density of the DAB.4HCl reaction product. 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. 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 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.

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. Diaminobenzidine appeared to be inactive in a carcinogenicity screen test using rats, but some individuals claim it may be a carcinogen.

Storage:

Storage at room temperature for short periods of time is acceptable. For long term storage, 0°C is recommended.

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 and Federal regulations. Material can also be treated by dissolving in copious quantities of household bleach, and after letting it stand overnight, it is flushed down the drain with plenty of water.