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DATASHEET

3,3-Diaminobenzidine (DAB) tetrahydrochloride

Product overview

Name Cat No Alternative names Biological description 3,3-Diaminobenzidine (DAB) tetrahydrochloride HB0687 DAB, DAB substrate <u>Overview</u>

DAB tetrahydrochloride is a water soluble form of DAB which is a derivative of benzene and is widely used in the staining of nucleic acids and proteins, particularly in IHC and HC procedures.

DAB is oxidized by hydrogen peroxide in the presence of peroxidases such as HRP. Once oxidized, DAB forms a dark brown insoluble precipitate at the reaction site to allow visualisation of the target.

This precipitate can be chelated with osmium tetroxide and the color produced can be enhanced by the addition of metals (e.g. nickel, copper, silver and cobalt).

Uses & applications

In IHC, DAB acts as a substrate for peroxidase enzymes conjugated to a primary or secondary antibody e.g. HRP-conjugated antibodies.

DAB is also used for peroxisome and mitochondrial COX activity staining and used to detect the presence and distribution of hydrogen peroxide in plants cells.

Biological action Purity Description DAB also enhances staining of iron. Dyes & stains >98% Chromogen staining agent widely used in IHC

Images



Biological Data

Application notes

DAB Staining using HRP Conjugated Secondary Antibodies

- 1% DAB (0.1g DAB, 10ml dH₂O, 3-5 drops of 10M HCl), mix well and ensure solution is an even brown color. This can be frozen and subsequently thawed
- 0.3% H₂O₂ (100µl of 30% H₂O₂, 10ml dH₂O), can be kept for short periods of time but better to make fresh

Working solution:

Mix a 1:1:20 ratio of 1% DAB: 0.3% H₂O₂: PBS (e.g. 250µl of 1% DAB solution with 250uL of 0.3% H₂O₂ and 5ml PBS which is sufficient for 100 sections)

Protocol:

- 1. Following secondary antibody incubation and washing add $50\mu l$ of DAB working solution to each section and incubate at room temperature for 10 minutes. During this time the color
- development can be monitored and the incubation time increased or decreased as required. 2. Rinse slides in running tap water for 5 minutes.
- 3. Counterstain with hematoxylin or proceed straight to dehydration and mounting.

Solubility & Handling

 Storage instructions
 -20°C

 Solubility overview
 Soluble in water (2% by mass)

 Important
 This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

Chemical Data

Chemical name Molecular Weight Chemical structure

Molecular Formula CAS Number PubChem identifier SMILES InChiKey MDL number Appearance 3,3'-Diaminobenzidine tetrahydrochloride 360.11 NH2

NHa 4HCI ŃНа

 $C_{12}H_{14}N_4.4HCl$ 868272-85-9 16211746 C1=CC(=C(C=C1C2=CC(=C(C=C2)N)N)N)N.O.Cl.Cl.Cl.Cl.Cl DXWSCXIZIHILNP-UHFFFAOYSA-N MFCD08273058Off-white to brown solid

References

3,3'-Diaminobenzidine staining interferes with PCR-based DNA analysis.

 Doelle et al (2018) Sci Rep. 8(1)

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van Duijin et al (201 3) J Histochem Cytochem 61(11) **PubMedID** 23887894

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