Hello Bio, Inc.
304 Wall St., Princeton, NJ 08540 USA

T. 609-683-7500
F. 609-228-4994

customercare-usa@hellobio.com

DATASHEET

3,3-Diaminobenzidine (DAB) tetrahydrochloride

Product overview

Name	3,3-Diaminobenzidine (DAB) tetrahydrochloride
Cat No	HB0687
Alternative names	DAB, DAB substrate
Biological description	**Overview**

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

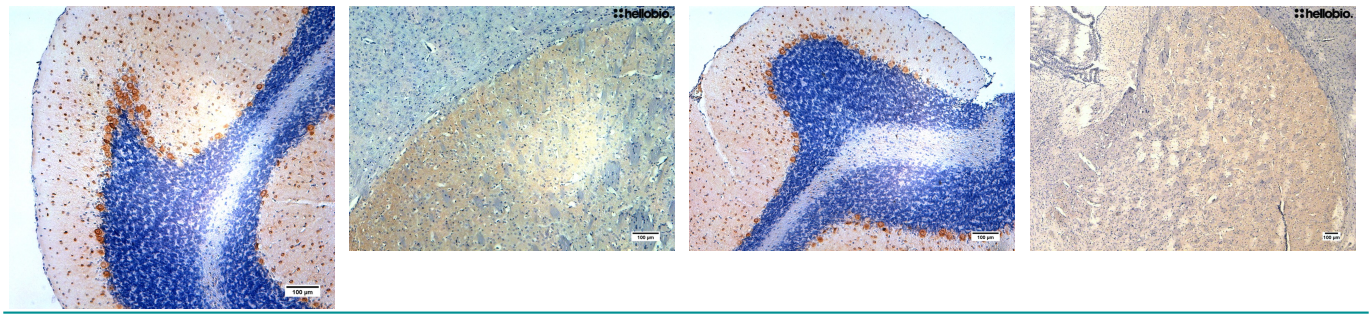
In IHC, DAB acts as a substrate for peroxidase enzymes conjugated to a primary or secondary antibody e.g. HRP-conjugated antibodies.

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.

DAB also enhances staining of iron.

Biological action	Dyes & stains
Purity	>98%
Description	Chromogen staining agent widely used in IHC

Images



Biological Data

Application notes	**DAB Staining using HRP Conjugated Secondary Antibodies**

Stock solutions:

- 1% DAB (0.1g DAB, 10ml dH_2O, 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% H_2O_2 (100µl of 30% H_2O_2, 10ml dH_2O), 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% H_2O_2 : PBS (e.g. 250µl of 1% DAB solution with 250uL of 0.3% H_2O_2 and 5ml PBS which is sufficient for 100 sections)

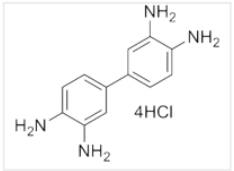
Protocol:

1. Following secondary antibody incubation and washing add 50µl 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	3,3'-Diaminobenzidine tetrahydrochloride
Molecular Weight	360.11
Chemical structure	
Molecular Formula	$C_{12}H_{14}N_4.4HCl$
CAS Number	868272-85-9
PubChem identifier	16211746
SMILES	C1=CC(=C(C=C1C2=CC(=C(C=C2)N)N)N)N.O.Cl.Cl.Cl.Cl
InChiKey	DXWSCXIZIHILNP-UHFFFAOYSA-N
MDL number	MFCD08273058
Appearance	Off-white to brown solid

References

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

Doelle et al (2018) Sci Rep. 8(1)
PubMedID 29352159

Detection of Hydrogen Peroxide by DAB Staining in Arabidopsis Leaves.

Daudi et al (2012) Bio Protoc 2(18)
PubMedID 27390754

Chromogen-based immunohistochemical method for elucidation of the coexpression of two antigens using antibodies from the same species.

Nakata et al (2012) J Histochem Cytochem 60(8)
PubMedID 22610462

Comparison of histological techniques to visualize iron in paraffin-embedded brain tissue of patients with Alzheimer's disease.

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

Comparison of histological techniques to visualize iron in paraffin-embedded brain tissue of patients with Alzheimer's disease.

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