

## DATASHEET

### 3,3-Diaminobenzidine (DAB) tetrahydrochloride

#### Product overview

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

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

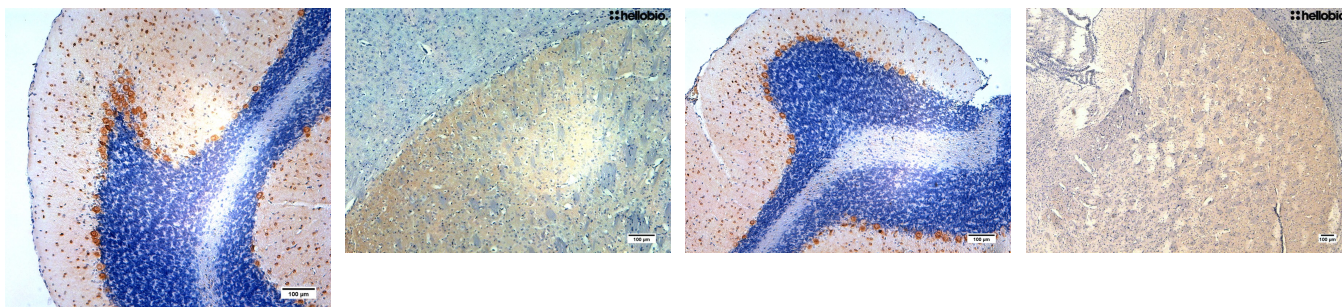
In IHC, DAB acts as a substrate for peroxidase enzymes conjugated to a primary or secondary antibody e.g. HRP-conjugated antibodies.

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.

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

#### Images



#### Biological Data

<b>Application notes</b>	DAB Staining using HRP Conjugated Secondary Antibodies
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Stock solutions:

- 1% DAB (0.1g DAB, 10ml dH<sub>2</sub>O, 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<sub>2</sub>O<sub>2</sub> (100µl of 30% H<sub>2</sub>O<sub>2</sub>, 10ml dH<sub>2</sub>O), 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<sub>2</sub>O<sub>2</sub> : PBS (e.g. 250µl of 1% DAB solution with 250uL of 0.3% H<sub>2</sub>O<sub>2</sub> 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.

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## Solubility & Handling

**Storage instructions**  
**Solubility overview**  
**Important**

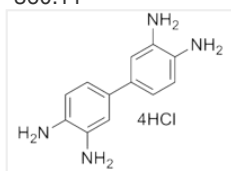
-20 °C  
Soluble in water (2% by mass)  
This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

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## Chemical Data

**Chemical name**  
**Molecular Weight**  
**Chemical structure**

3,3'-Diaminobenzidine tetrahydrochloride  
360.11



**Molecular Formula**  
**CAS Number**  
**PubChem identifier**  
**SMILES**  
**InChiKey**  
**MDL number**  
**Appearance**

C<sub>12</sub>H<sub>14</sub>N<sub>4</sub>·4HCl  
868272-85-9  
16211746  
C1=CC(=C(C=C1C2=CC(=C(C=C2)N)N)N)N.O.Cl.Cl.Cl.Cl  
DXWSCXIZIHILNP-UHFFFAOYSA-N  
MFCD08273058  
Off-white to brown solid

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## 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 (2013) 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.**

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[23887894](#)

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