

# DAB, HRP chromogenic ins.substrate

## Description

High quality DAB - for immunoblotting and immunohistochemical staining techniques

**cat.number :** [UP01012G](#) , 5g [UP01012H](#) , 10g

**Name:** **DAB** : 3,3' Diamino Benzidine Tetrahydrochloride  
 $C_{12}H_{14}N_4N_2 \cdot (HCl)_4$  ; 360.12  
Biotech grade

**Storage:** Frozen, keep dry, protect from light (M)  
Warm to room temperature before opening

### Technical tips:

DAB, 3,3' Diaminobenzidine is a widely used chromogenic substrate for Peroxidase (HRP) detections. Our DAB solution contains 0.0694 M DAB of highest purity, and proprietary agents for enhancement of sensitivity and stability. It is a 50x concentrate that is easily diluted with the provided buffer (supplied as 10x concentrate, pH 7.6 when diluted) before use on immunoblots and slides that have been probed with HRP labelled probes.

**Security note:** DAB is a suspected carcinogen. R36/37/38-45-42/43 S:36/37/39

## Directions for use

### Protocol 1 : DAB as HRP substrate in IHC technique

Use gloves should be worn when handling DAB, as well other harmful reagents required in the procedure..

- **Sample and Slides preparation:**

Fix (i.e. with 10% formal calcium)

Embed with paraffin

Cut in 3-10µm thick sections

Mount on chromic acid etched and poly-L-lysine or chrome alum/gelatine coated slides.

Dry at 37°C for at least 1 hour; overnight is preferable

- **Prepare TBS buffer** [0.005M TRIS/HCl buffered saline, pH 7.6]: reconstitute TBS #[GS4200](#)

Home made TBS: weigh out NaCl 81g , TRIS 6g , dissolve in distilled water 10Liters, and adjust pH with HCl 1N soln (ca42ml).

- **prepare DAB Solutions**

Note: DAB is a possible carcinogen; wear gloves when using it. The dry powder must never be used on the open bench; always use a fume cupboard.

Prepare Soln. A: 280ml. TBS.

Prepare Soln. B: 0.15g 3',3'diaminobenzidine tetrahydrochloride in 10ml TBS.

Prepare Soln. C: 0.20g imidazole in 10ml TBS.

CAUTION: these solution are stable, but step should be performed prior to use for DAB staining (see below DAB staining/step 1.)

Mix solns. A, B and C immediately prior to use.

Incubate sections for 30 secs., add 225 microlitres of 20 vol. hydrogen peroxide (#[15983Q](#)) and incubate for a further 10 mins (see below DAB staining/step 1.).

CAUTION: Hydrogen peroxide is a powerful oxidizing agent; avoid skin contact.

- **Stain the slide with HRP probe**

Contact your local distributor Uptima, powered by

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Perform immunodetection with i.e. a I antibody then a secondary antibody. Signal amplification can be achieved with Peroxidase anti Peroxidase (PAP) reagent. Incubate each reagent for 30-60min, and wash 2-3 times with TBS for 3 min. At the end of the staining procedure, wash well with TBS.

Dilute primary and biotinylated secondary antisera as appropriate in 1:20 normal irrelevant serum (same species as II Ab). Do not allow the sections to dry out at any stage of the detection and DAB staining.

- **Staining with DAB**

1. Incubate in DAB solution for 10 mins (see above).
2. Wash in running tap water.
3. Incubate in 0.5% copper sulphate in 0.9% sodium chloride for 10 mins. Wash in running tap water. The HRP probed antigen appear in black/brown.

- **Post staining, Mounting...**

Counterstaining can be performed with haematoxylin (nuclei in blue) or other suitable counterstain. Dehydrate, clear. Mount sections (DPX, Histochoice,...)

## Other protocols with DAB as HRP substrate

Other protocols can be found in the literature for IHC, blotting... Inquire.

[Double in situ Labeling of Embryos](#)

[Double Immunohistochemistry](#)

## Related reagents

See our our [BioScience catalog](#) for:

\*other reagents for immunodetections with DAB substrate:

Hydrogen Peroxide [#15983Q](#)

WB:HP-labeled secondary antibodies (p.A324)

HRP labeled (strept)avidins (A350)

ProTran 0.2µm NC membranes, 20x20cm, [#S31441](#)

Blotting paper 1mm thick, 460x570cm, [#BP2791](#)

Stains: Protein Membrane Reversible stain [#20078A](#)

buffers and saturants (A365):

SeaBlock agent [#UP40301A](#)

TBS with non fat milk [#GS4160](#)

TBS with Tween20 [#GS4200](#)

BSA [#UPQ84170](#) (powder) or [#UP900130](#) (soln 30%)

BioBlock Saturating agent for blotting (inTBS) [#N13650](#)

Non fat milk powder [#768701](#)

\*Other substrates for HRP: chemiluminogenic:

UptiLight Classic [#UP996190](#), spray [#BM4961](#), UltraSensitive [#58372A](#)

chromogenic:

op-Metal Enhanced DAB kit [#679921](#)

DAB tablets [#UP732310](#)

Contact uptima – Interchim for any question

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