



## DAB Substrate Kit High Contrast

**REF** / Cat. No.: DAB500plus 500 tests, 55 ml  
DAB5000plus 5000 tests, 500 ml

### Instructions for use

#### Intended use

DAB Substrate kit High Contrast is developed for immunohistochemical and *in situ*-hybridisation staining procedures with horse radish peroxidase (HRP). DAB (3,3'-Diaminobenzidine) leads to the formation of a brown precipitate at the location of the target antigen or target nucleic acid. The precipitate is insoluble in aqueous and organic solvents and can be observed by light microscopy.

The kit is especially useful when a high contrast between chromogen and counter stain is desired. Compared to standard DAB staining systems the DAB Substrate High Contrast kit gives a darker brown colour and a higher sensitivity.

DAB Substrate Kit High Contrast is intended for in vitro diagnostic use.

#### Reagents provided

##### **REF** / Cat. No. DAB500plus

3 ml DAB Chromogen (liquid DAB concentrate)  
11 x 5 ml DAB Substrate Buffer High Contrast

##### **REF** / Cat. No. DAB5000plus

30 ml DAB Chromogen (liquid DAB concentrate)  
500 ml DAB Substrate Buffer High Contrast

#### Storage and Handling

The solutions should be stored at 2-8°C without further dilution. Please store the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the expiry date indicated on the label. Do not use product after the expiry date.

The working solution should be prepared freshly at the day of use. Once the two reagents are combined, the resulting solution is stable for up to six hours. Excess working solution should be disposed as hazardous substance.

A positive and a negative control have to be carried out in parallel to the test material. If you observe unusual staining or other deviations from the expected results which could possibly be caused by the kit reagents please contact Zytomed Systems' technical support or your local distributor.

#### Precautions

Use by qualified personnel only. The DAB chromogen is hazardous to your health. Wear protective clothing to avoid contact of reagents or specimen with eye, skin or mucous membrane. In case of a reagent or specimen coming into contact with a sensitive area, wash the area with large amounts of water. Microbial contamination of the reagents must be avoided, since otherwise non-specific staining may occur.

Material safety data sheets (MSDS) are available upon request.

#### Reagent preparation (preparation of the working solution)

##### DAB500plus:

Add 5 drops of DAB Chromogen (DAB concentrate) to one bottle of DAB Substrate Buffer High Contrast and mix thoroughly.

##### DAB5000plus:

Add 50 µl of DAB Chromogen (DAB concentrate) to 1 ml of DAB Substrate Buffer High Contrast and mix thoroughly.

*Note: Typical working concentrations are 50 µl (0.9 mg) DAB per ml substrate buffer. The colour intensity can be adjusted by decreasing or increasing the DAB concentration in the working solution. Maximum sensitivity in immunohistochemical staining can be achieved by working concentrations of about 80 µl (1.5 mg) DAB per ml substrate buffer.*

## Staining procedure

- 1) Rinse the slide with wash buffer after the previous incubation step.
  - 2) Apply the DAB High contrast working solution to the slide. Incubate for 5-15 minutes.
  - 3) Rinse with distilled H<sub>2</sub>O.
  - 4) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity).
  - 5) Rinse with distilled H<sub>2</sub>O.
  - 6) Blueing in tap water for at least 5 minutes.
  - 7) Dehydrate through a graded series of ethanol and clear in xylene. Mount with a permanent mounting medium.
- Note: It is also possible to mount DAB High Contrast with aqueous mounting media.

## Quality control

We recommend carrying out a positive and a negative control with every staining run. The positive control permits the validation of appropriate processing of the sample. If the negative control has a positive result, this points to unspecific staining. Please refer to the instructions of the detection system for guidance on general quality control procedures.

## Troubleshooting

If you observe unusual staining or other deviations from the expected results please read these instructions carefully, contact Zytomed Systems' technical support or your local distributor. Also refer to the instructions of the detection systems for guidance on general troubleshooting.

## Expected results

During the reaction of the substrate with horse radish peroxidase in presence of the chromogen DAB, a brown precipitate is formed at the location of the target antigen or nucleic acid. The precipitate is insoluble in aqueous and organic solvents and can be observed by light microscopy.

## Limitations of the procedure

Immunohistochemistry is a complex method in which histological as well as immunological detection methods are combined. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). In some tissues endogenous peroxidase activity may cause non-specific staining. The enzyme activity should be blocked by incubation with hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub> solution, Cat. No. ZUC019). The step is carried out before incubation with primary antibody but after dewaxing and rehydration.

Background staining due to endogenous biotin can be blocked through an avidin-biotin blocking step prior to the primary antibody incubation step. Inadequate counterstaining and mounting can influence the interpretation of the results.

Zytomed Systems guarantees that the product will meet all requirements described from its shipping date until its expiry date, as long as the product is correctly stored and utilized. No additional guarantees can be given. Under no circumstances shall Zytomed System be liable for any damages arising out of the use of the reagent provided.

## Performance characteristics

Zytomed System has conducted studies to evaluate the performance of the kit reagents in combination with standard detection systems. The product has been found to be suitable for the intended use.

## Bibliography








Elias JM "Immunohistopathology – A practical Approach to Diagnosis" ASCP Press 2003  
 Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983

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Doc: DBE\_DAB500plus\_5000plus

Explanation of the symbols on the product label:

<b>REF</b>	Bestellnummer Catalog Number Reference du catalogue		Verwendbar bis Use By Utiliser jusque			Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation
<b>LOT</b>	Chargenbezeichnung Batch Code Code du lot		Lagerungstemperatur Temperature Limitation Limites de température		<b>RUO</b>	Nur für Forschungszwecke For Research Use Only Pour la recherche uniquement
<b>IVD</b>	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro		Achtung/Gefahr Warning/Danger Attention/Danger			Hersteller / Manufacturer / Fabricant Zytomed Systems GmbH Anhalternerstraße 16 14163 Berlin, Germany Tel: (+49) 30-804 984 990 www.zytomed-systems.de
	Achtung/Gefahr Warning/Danger Attention/Danger		Achtung Warning Attention	