

DAB High Contrast Kit

Reference: AP13004



1 of 2

INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP13004. 55 mL / 500 Tests.

SUMMARY, EXPLANATION AND LIMITATIONS:

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.

Immunohistochemistry (IHC) is a complex technique in which immunological and histological detection methods are combined. In general, the manipulation and processing of tissues before immunostaining, especially different types of tissue fixation and embedding, as well as the nature of the tissues themselves may 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 (H₂O₂ solution, REF AP12002/AP12003). 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.

APPLICATIONS:

DAB Substrate kit High Contrast is intended for immunohistochemical and in situ-hybridisation staining procedures with HRP.

The interpretation of the stain results is the full responsibility of the user. Any experimental result must be confirmed by a medically established diagnostic product or procedure.

REAGENT PROVIDED:

3 ml **DAB Chromogen** (liquid DAB concentrate)

11 x 5 ml **DAB Substrate Buffer High Contrast**

METHOD AND PROCEDURE:

Principle of the method: The IHC as technique to demonstrate the presence of an antigen in tissues and cells, is a sequential procedure of several steps: the application of antibody specific for the antigen of interest (primary antibody), the detection and visualization of bound antibody by one of a variety of enzyme chromogenic systems and washing steps. The chromogenic enzyme activation results in a visible product at the site where the antigen is located. The results can be evaluated in a light microscope.

DAB leads to the formation of a dark-brown precipitate at the location of the target antigen or target nucleic acid and can

be observed by light microscopy. DAB High Contrast kit is especially useful when a high sensitivity is desired.

Specimen: Formalin-fixed paraffin-embedded tissue section.

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

Procedure:

- 1) Rinse the slide with wash buffer after the previous incubation step.
- 2) Apply the DAB working solution onto the slide. Incubate for 5-15 minutes.
- 3) Rinse with distilled H₂O.
- 4) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity).
- 5) Rinse with distilled H₂O.
- 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 with aqueous mounting media.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for IHC procedures are not provided with this product. This includes adhesive slides and cover slips, positive and negative control tissues, Xylene or adequate substitute, ethanol, distilled H₂O, heat pretreatment equipment (pressure cooker, steamer, microwave), pipettes, Coplin jars, glass jars, moist chamber, histological baths, negative control reagents, counter-staining solution, mounting materials, and microscope.

Buffered solutions for antigen retrieval, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Genova Scientific.

STORAGE AND STABILITY:

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 can be used for up to six hours. Excess working solution needs to be disposed as hazardous substance. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user. Genova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances is Genova Scientific obliged to cover damages caused by use of this reagent.



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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2 of 2

TROUBLESHOOTING:

If unusual staining is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the detection system. If this does not solve the problem, please contact Genova Scientific's technical support department or your local distributor.

PRECAUTIONS:

Use only by qualified personnel.
Some of the reagents used in this kit are 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.

PERFORMANCE CHARACTERISTICS:

Genova Scientific has performed studies to evaluate the functioning of the kit for use with standard detection systems, concluding that 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, Morales AR. Immunoperoxidase, part 1: the techniques and its pitfall. Lab Med 1983; 14:767-770.

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