Product datasheet **MON-APP177**

Monosan DAB Substrate Kit (Brown), 5.000 tests

Reagents

Instructions for use

Product name	Monosan DAB Substrate Kit (Brown), 5.000 tests
Intended Use	DAB Substrate kit is intended 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.
Applications	IHC-P, IHC-Fr, IF
Summary and explanation	-

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

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Principle of method -

Reagents provided

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

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Storage and handling

The solutions should be stored at 2-8°C without fur ther 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. 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 our

Reagent preparation

Add 50 µl DAB Chromogen (DAB concentrate) to 1 ml of DAB Substrate Buffer 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.

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.

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.

Trouble shooting

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

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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.

Performance

Studies have been conducted to evaluate the performance of the kit reagents. The product has been found to be suitable for the intended use

Limitations of 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 (H2O2 solution). 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. Sanbio 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 Sanbio be liable for any damages arising out of the use of the reagent provided.

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.

References

1. Elias JM Immunohistopathology – A practical Approach to Diagnosis ASCP Pr
2 Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-139, 1983
3. -

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