Product datasheet **MON-APP173**

Monosan Permanent AEC Kit, 200 ml

Reagents

Instructions for use

Product name	Monosan Permanent AEC Kit, 200 ml
Intended Use	Permanent AEC Kit is intended for immunohistochemical and in situ-hybridisation staining procedures with horse radish peroxidase (HRP). AEC (3-Amino-9-ethylcarbazol) leads to the formation of a red-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. Permanent AEC Kit is intended for research use only.
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

5.5 ml Reagent 1
3 ml Reagent 2
3 ml Reagent 3 (Chromogen)
4.5 ml Reagent 4 (H_2O_2)
1 Dilution Vial

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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. Excess working solution should be disposed. 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 technical support.

Reagent preparation

1) Pipette 5 ml distilled or deionised water into the provided dilution vial.
2) Add 3 drops buffer concentrate (Reagent 1). Mix thoroughly.
3) Add 2 drops Reagent 2. Mix thoroughly.
4) Add 2 drops AEC chromogen (Reagent 3). Mix thoroughly.
5) Add 2 drops H2O2 substrate (Reagent 4). Mix thoroughly.
This working solution is stable for at least 16 hours if stored at 2-8°C in a dark place.

Procedure

1) Apply the Permanent AEC working solution onto the slide. Incubate for 5-15 minutes. (Incubation time can be extended, if desired.) 2) Rinse with distilled or deionised H2O. 3) Counterstain with haematoxylin for about 30 seconds up to 5 minutes (depending on the desired staining intensity). 4) Rinse with distilled or deionised H2O. 5) Blueing in tap water for at least 5 minutes. 6) Dehydrate through a graded series of ethanol and clear in xylene. Mount with a permanent mounting medium.

Expected results

During the reaction of the substrate with horse radish peroxidase in presence of the chromogen AEC, a red-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. A longer exposure to absolute ethanol can result in decreasing staining intensity. Use of recycled alcohol to dehydrate tissue slides after staining is not recommended. 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

Precautions

Use by qualified personnel only. 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. A material safety data sheet (MSDS) is 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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