Product datasheet **MON-APP185**

Monosan Permanent AP-Red Kit, 1 Kit (125 ml/1,250 tests)

Reagents

Instructions for use

Product name	Monosan Permanent AP-Red Kit, 1 Kit (125 ml/1,250 tests)
Intended Use	Permanent AP Red Kit is developed for immunohistochemical and in situ-hybridisation staining procedures with alkaline phosphatase. Permanent AP Red leads to the formation of a magenta-red 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 or fluorescence microscopy.
Applications	IHC-P, IHC-Fr, IF
Summary and explanation	-

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Principle of method -

Reagents provided

125 ml Permanent AP Red Buffer
2 ml Permanent AP Red Chromogen
1 Dilution Vial

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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 prepared is stable for about 60 minutes and should therefore be used directly after preparation. Excess working solution should be discarded. 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

Reagent preparation (Preparation of the working solution) 1) Pipette 2.5 ml AP Red Buffer into the provided dilution vial and let it come to room temperature. The chromogen should still be kept cool. 2) Directly prior to use add 1 drop of Permanent AP Red Chromogen into the buffer. Mix thoroughly. 3) The solution is stable for about 60 minutes. Preparation should be done directly before use. Make sure to pipet the chromogen/substrate mix on the last slide of the staining run within 40 min after mixing. If you want to prepare other quantities of the working solution, please use same ratio AP Red Buffer and Chromogen

Procedure

1) Rinse the slide with wash buffer after the previous incubation step. 2) Apply freshly prepared Permanent AP Red working solution onto the slide. Incubate for 10 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 Permanent AP Red with aqueous mounting media.

Expected results

During the reaction of the substrate with alkaline phosphatase in presence of the chromogen Permanent AP Red, a magenta-red 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 or fluorescence microscopy (Texas Red filter).

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 alkaline phosphatase activity may cause non-specific staining. However, neither intestinal nor placental alkaline phosphatase can be blocked with levamisole. Therefore, tissues of this origin should be stained with peroxidase detection systems. A higher sensitivity can be obtained when a second chromogenic substrate step is used (i. e. 2 x 10 min Permanent AP Red). 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. 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. 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 might appear. 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

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