Product datasheet **MON-APP210**

Permanent HRP Green, 1 kit (100 ml)

Reagents

Instructions for use

Product name	Permanent HRP Green, 1 kit (100 ml)
Intended Use	Permanent HRP Green Kit is intended for immunohistochemical and in situ-hybridisation staining procedures with horse radish peroxidase (HRP). It results in the formation of a green precipitate at the location of the target antigen or target nucleic acid. The precipitate is insoluble in organic solvents and can be observed by light microscopy. The HRP Green precipitate shows a very good contrast to red chromogenic substrates used with alkaline phosphatase detection systems and is therefore especially recommended for double stains. Permanent HRP Green 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

100 ml HRP Green Substrate Buffer
3 ml HRP Green Chromogen
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. It is stable for at least 4 hours. 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 1 ml HRP Green Substrate Buffer into the provided dilution vial. 2) Add 1 drop (30 µl) of HRP Green Chromogen. Mix thoroughly. 3) The resulting working solution is stable for at least 4 hours. If you want to prepare other quantities of the working solution, please use the same ratio HRP Green Substrate and HRP Chromogen.

Procedure

1) Rinse the slide with wash buffer after the previous incubation step. 2) Apply the Permanent HRP Green working solution onto the slide. Incubate for 2-10 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 5 minutes. 7) Dehydrate through a graded series of ethanol and clear in xylene. Do not exceed incubation times of 30 sec per dehydration step. Use only high purity xylene. Mount with a permanent mounting medium. Note: The colour intensity can be intensified by increasing the chromogen concentration (up to 3 drops or 90 µl chromogen) in the working solution. Lithium carbonate may have a negative effect on the staining result. We recommend to only bluing in tap water. Occasionally precipitates may appear in the HRP Green Chromogen solution. This doesn’t affect the staining result.

Expected results

During the reaction of the substrate with horse radish peroxidase in presence of the Permanent HRP Green chromogen, a green precipitate is formed at the location of the target antigen or nucleic acid. The precipitate is insoluble in 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, 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

Too long incubation steps at the final dehydration serious can diminish the staining intensity. Also low grade xylene and some forms of recycled alcohol can have a negative effect on the staining result. In double stain procedures we recommend to use Permanent HRP Green as the last chromogen. 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). 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 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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