

Permanent AP-Red Kit

Reference: AP13100



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INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP13100. 125 mL / 1250 test

SUMMARY, EXPLANATION AND LIMITATIONS:

Permanent AP Red Kit is developed for immunohistochemical and *in situ*-hybridisation staining procedures with alkaline phosphatase (AP). 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.

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 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 (i.e. AP11345). 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.

APPLICATIONS:

Permanent AP Red Kit is intended for immunohistochemical and *in situ*-hybridisation staining procedures with AP. 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:

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

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.

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).

Specimen: Formalin-fixed paraffin-embedded tissue section.

Reagent preparation: 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.

If you want to prepare other quantities of the working solution, please refer to the examples in this table:

Buffer	Chromogen	Buffer	Chromogen
0,5 mL	8 µL	5 mL	80 µL
1 mL	16 µL	10 mL	160 µL
2 mL	32 µL	15 mL	240 µL
3 mL	48 µL	20 mL	320 µL
4 mL	64 µL	25 mL	400 µL

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.

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 Gennova 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 prepared



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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is stable for about 60 minutes and should therefore be used directly after preparation. Excess working solution should be discarded. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.

Gennova 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 Gennova Scientific obliged to cover damages caused by use of this reagent.

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 Gennova 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:

Gennova 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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