GN Plus AP Polymer Detection System Mouse/Rabbit/Rat

Reference: AP11346

INTENDED USE AND PRESENTATION:
For *in vitro* diagnostic use.
AP11346. 50 mL / 500 Tests.

SUMMARY, EXPLANATION AND LIMITATIONS:
The purpose of the immunohistochemical staining is to make tissue and cell antigens visible. GN Plus AP Polymer anti-Mouse/Rabbit/Rat is a highly sensitive detection reagent intended for use in immunohistochemistry and immunocytochemistry. The enzyme polymer consists of several molecules of secondary antibodies covalently bound to several molecules of alkaline phosphatase (AP). Visualisation occurs via an enzyme-substrate reaction in the presence of a colorising reagent which permits microscopical analysis. The test system is suitable for the detection of mono- and polyclonal primary antibodies and sera obtained from mice, rabbit or rat. In contrast to other detection techniques, which often use the streptavidin-biotin system the GN Plus AP Polymer kits avoid the problem of background staining caused by endogenous biotin in the tissue.
GN Plus AP Polymer anti-Mouse/Rabbit/Rat Kit is designed for the qualitative detection of antigens in fixed paraffin-embedded tissue sections, in frozen tissue sections, and in cytological samples. It was developed for use in combination with mono- and polyclonal primary antibodies and sera obtained from mice, rabbit or rat. The kit can be used for examining tissues fixed in different solutions, e.g. formalin (neutrally buffered), B5, Bouin, ethanol, or HOPE.
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). Inadequate counterstaining and mounting can influence the interpretation of the results. The colour intensity of the reaction product can decrease with time, especially when exposed to light.

APPLICATIONS:
GN Plus AP Polymer anti-Mouse/Rabbit/Rat Kit can be used for the qualitative detection of antigens in fixed paraffin-embedded tissue sections, in IHC technique. It was developed for use in combination with mono- and polyclonal primary antibodies and sera obtained from mice, rabbit or rat.
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:
50 mL **Blocking Solution,** Ready to use.
50 mL **Post-Block,** Ready to use.
50 mL **AP-Polymer,** Ready to use.
2 x 50 mL **Buffer AP Red Permanent,** Ready to use.
2 x 0.8 mL **Chromogen AP Red Permanent,** Concentrated.

METHOD AND PROCEDURE:
Principle of the method: Paraffin-embedded tissue sections are first deparaffinised and rehydrated. Background staining caused by unspecific binding of the primary antibody or the secondary antibody in the AP polymer is minimized by incubation with a protein blocking solution ("Blocking Solution", provided with this kit). This step can be omitted if the primary antibodies are diluted in an appropriate buffer.
The next step is incubation with the specific primary antibody. After washing, the enhancement reagent ("PostBlock") is applied and incubated. A second washing is followed by the application of the AP-polymer. Any excess of unbound AP polymer is thoroughly washed away after incubation. The addition of the chromogenic substrate starts the enzymatic reaction of the alkaline phosphatase which leads to colour precipitation where the primary antibody is bound.
The colour can be observed with a light microscope. The chromogen used determines the colour. The chromogen Fast Red leads to the formation of a magenta-red product of reaction at the place of the target antigen. Other suitable chromogens are Permanent AP Red (magenta-red), New Fuchsin (magenta-red) or NBT (blue-black) with its substrate BCIP.
Specimen: Formalin-fixed paraffin-embedded tissue section.
Reagent preparation: Reagents should be at room temperature when used.
- Deparaffinise and rehydrate paraffin-embedded tissue sections.
- Pre-treatment (optional) with HIER (Heat Induced Epitope Retrieval) or enzymatic digestion.
- Tissue sections have to be completely covered with the different reagents in order to avoid drying out.

Staining Procedure:
1. Blocking Solution (protein block) (This step is optional.) 5 min.
2. Washing with wash buffer 1 x 2 min.
3. Primary antibody (optimally diluted) or negative control reagent 30-60 min.
4. Washing with wash buffer 3 x 5 min.
5. PostBlock (yellow) 20 min.
6. Washing with wash buffer 3 x 5 min.
7. AP-polymer (red) 30 min.
8. Washing with wash buffer 3 x 2 min.
9. Permanent AP Red, Fast Red, NBT/BCIP or New Fuchsin 5-15 min. (Controlling the colour intensity via light microscope is recommended.)

Preparation of Permanent AP Red working solution (included):
a) 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.
b) Directly prior to use add 1 drop of Permanent AP Red Chromogen into the buffer. Mix thoroughly.
c) 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

10. Stopping the reaction with distilled H2O when the desired colour intensity is attained
11. Counterstaining and blueing
12. Mounting: permanent with Permanent AP Red, NBT/BCIP or New Fuchsin, aqueous with Fast Red.

See our web site at www.gennova-europe.com for detailed protocols ancillary reagents and support products.

EXPECTED RESULTS:
During the reaction of the substrate with alkaline phosphatase in the presence of a chromogen, a coloured precipitate is formed at the location of the bound primary antibody. This reaction only takes place if the target antigen is existent in the tissue. The chromogen used determines the colour of the precipitate. The analysis is carried out using a light microscope.

REQUIRED MATERIALS BUT NOT SUPPLIED:
All reagents, materials, and laboratory equipment for IHC procedures are not provided with this product. This includes antibodies, adhesive slides and cover slips, positive and negative control tissues, Xylene or

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adequate substitute, ethanol, distilled H_2O, 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.
Antibodies, buffered solutions for antigen retrieval, enzyme treatments, others highly sensitive detection systems, and other auxiliary reagents are available from Gennova Scientific.

STORAGE AND STABILITY:
Store at 2-8 °C without further dilution. Please store the reagent in a dark place and do not freeze it. Do not use after the expiration date. 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:
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 read these instructions carefully. If this does not solve the problem, please contact Gennova Scientific's technical support department or your local distributor.
No staining on an actually positive control slide:
1 Reagents were not used in the proper order.
2. Chromogenic substrate solution was too old.
3. Bleaching because chromogen and mounting medium are incompatible.
4. The antigen/epitope in the tissue was insufficiently accessible to the primary antibody. Try a pre-treatment such as heat pre-treatment or
enzyme digestion. If you used a pre-treatment it should be extended.
5. Primary antibody not from mouse or rabbit, but from a different species.
6. The antigen/epitope was not stable in the fixation and/or pre-treatment procedure used. Try another fixation or pre-treatment.
Weak staining:
1. Inadequate fixation or overfixation.
2. Incomplete deparaffinisation.
3. The antigen/epitope in the tissue was insufficiently accessible to the primary antibody. If you used heat pre-treatment or enzyme digestion it should be extended.
4. Excessive incubation with Blocking Solution or insufficient washing after this step.
5. Too much wash buffer remains on the slides after washing, diluting the reagents applied in the next step.
6. If you are using PBS-based wash buffer: the activity of alkaline phosphatase in the reagents is blocked if too much wash buffer remains on the slides.
7. Incubation times were too short or primary antibody concentration too low.
8. Chromogenic substrate solution was too old.
Nonspecific background staining or overstaining:
1. Incomplete deparaffinisation.
2. Excessive tissue adhesive on slides.
3. Insufficient washing especially after the incubation with the enzyme polymer or the chromogenic substrate solution. These washings are critical.
4. Tissue was allowed to (partially) dry out with reagents on.
5. Unspecific binding of the primary antibody. Please use the Blocking Solution provided with this kit or dilute the primary antibody in appropriate diluents.
6. Incubation time of the primary antibody was too long or primary antibody concentration too high.
7. Incubation time of the chromogenic substrate solution was too long or reaction temperature too high (e.g. if temperature in the laboratory is high).
8. The substrate is metabolised by endogenous alkaline phosphatase in the tissue. This undesired activity can often be suppressed using levamisole (see section Limitations of the Procedure).

PRECAUTIONS:
Use by qualified personnel only.
Wear protective clothing to avoid eye, skin or mucous membrane contact with the reagents. In case of a reagent coming into contact with a sensitive area, wash the area with large amounts of water. ProClin 300 and sodium azide (NaN3), used for stabilisation, are not considered hazardous materials in the concentrations used. Sodium azide deposits in drainage pipes made of lead or copper can result in the formation of highly explosive metallic azides. To avoid such deposits in drainage pipes, sodium azide should be discarded in a large volume of running water. Material safety data sheets (MSDS) for the pure substances are available upon request. Microbial contamination of the reagents must be avoided, since otherwise non-specific staining might appear.

PERFORMANCE CHARACTERISTICS:
Gennova Scientific has conducted studies to evaluate the performance of the kit reagents. 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.

REF	Catalog number	LOT	Batch code	IVD	In Vitro diagnostic medical device
	Temperature limitation		Expiration date	Σ	Test number
	Manufacturer		See instruction for use		

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