GN Plus HRP Polymer Detection System Mouse/Rabbit/Rat

Reference: AP11345

INTENDED USE AND PRESENTATION:
For *in vitro* diagnostic use.
AP11345. 50 mL / 500 Tests.

SUMMARY, EXPLANATION AND LIMITATIONS:
The purpose of the immunohistochemical staining is to make tissue and cell antigens visible. GN Plus HRP 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 horseradish peroxidase (HRP). 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 HRP Polymer kits avoid the problem of background staining caused by endogenous biotin in the tissue.
GN Plus HRP 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). In some tissues endogenous peroxidase activity may cause non-specific staining. The enzyme activity should be blocked by incubation with hydrogen peroxide solution (H_2O_2 solution, REF AP12002/AP12003). 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.

APPLICATIONS:
GN Plus HRP 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 **HRP Polymer anti-Mouse/Rabbit/Rat** (RTU)
2 x 50 ml **DAB Substrate Buffer**
2 x 3 ml **DAB Chromogen** (Concentrated)

METHOD AND PROCEDURE:
Principle of the method: Paraffin-embedded tissue sections are first deparaffinised and rehydrated. Endogenous peroxidase activity in the tissue may cause non-specific staining. This enzyme activity can be blocked by incubation with 3% H_2O_2-solution (peroxide block). Background staining caused by unspecific binding of the primary antibody or the secondary antibody in the HRP Polymer is minimized by incubation with a protein blocking solution. 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 GN Plus HRP Polymer is applied and incubated. Any excess of unbound polymer is thoroughly washed away after incubation. The addition of the chromogenic substrate starts the enzymatic reaction of the peroxidase 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 DAB forms a dark brown precipitate product of reaction at the place of the target antigen. The chromogen AEC leads to the formation of a red-brown.
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. Peroxide blocking (3 % H_2O_2 solution) 10 min.
2. Washing with wash buffer 1 x 2 min.
3. Blocking Solution (This step is optional.) 5 min.
4. Washing with wash buffer 1 x 2 min.
5. Primary antibody (optimally diluted) or negative control reagent 30-60 min.
6. Washing with wash buffer 3 x 5 min.
7. HRP Polymer anti-Mouse/Rabbit/Rat 30 min.
8. Washing with wash buffer 3 x 2 min.
9. Apply the DAB working solution onto the slide. Incubate for 5-15 minutes. (Controlling the colour intensity via light microscope is recommended).

DAB working solution preparation: Add 1 drops of DAB Chromogen (DAB concentrate) to 1 ml of DAB Substrate Buffer and mix thoroughly. The working solution should be prepared freshly and light protected. Once the two reagents are combined, the resulting solution can be used for up to six hours.
10. Stopping the reaction with distilled H_2O when the desired colour intensity is attained.
11. Counterstaining and blueing.
12. Mounting: permanent with DAB.

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 horseradish peroxidase 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 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.

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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. If fresh solutions are required, these must be prepared immediately prior to use, and will be stable for at least 6 hours if stored at in a dark place. Unused portion of working solution should be discarded after this time. 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:

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, rabbit or rat 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. Incubation times were too short or primary antibody concentration too low.
7. 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 Blocking Solution 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 horse radish peroxidase in the tissue. Maybe the hydrogen peroxide solution used for blocking was inactivated.

PRECAUTIONS:

Use only by qualified personnel.

Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific staining results. Material safety data sheet (MSDS) is available upon request.

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:

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REF Catalog number | LOT Batch code | IVD In Vitro diagnostic medical device

Temperature limitation | Expiration date | Test number

Manufacturer | See instruction for use

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