

GN Plus AP-Fast Red Kit, Broad Spectrum

Reference: AP11500



1 of 2

INTENDED USE AND PRESENTATION:

For *in vitro* diagnostic use.

AP11500. 8 mL / 80 Tests.

SUMMARY, EXPLANATION AND LIMITATIONS:

The purpose of the immunohistochemical staining is to make tissue and cell antigens visible. GN Plus AP-Fast Red Kits, Broad Spectrum is a highly sensitive detection kit intended for use in immunohistochemistry and immunocytochemistry. The method is based on the streptavidin-biotin system which means that a biotinylated secondary antibody binds to several molecules of a conjugate composed of streptavidin and alkaline phosphatase. Visualisation occurs via an enzyme-substrate reaction in the presence of a colouring reagent which permits microscopical analysis. The biotinylated secondary antibody in GN Plus AP-Fast Red Kit, Broad Spectrum is polyvalent. With this kit it is therefore possible to detect mono- and polyclonal primary antibodies and sera obtained from mouse, rabbit, rat, and guinea pig.

GN Plus AP-Fast Red Kit, Broad Spectrum is based on the streptavidin-biotin system. It is designed for the qualitative detection of antigens in fixed paraffin-embedded tissue sections, in frozen tissue sections, and in cytological samples. The kit is developed for use in combination with mono- and polyclonal primary antibodies and sera obtained from mouse, rabbit, rat, and guinea pig. The GN Plus AP-Fast Red Kit, Broad Spectrum can be used for examining tissues fixed in different solutions, e.g. formalin (neutrally buffered), B5, Bouin, ethanol, or HOPE.

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). Endogenous alkaline phosphatase activity or the endogenous biotin content may cause non-specific staining. The enzyme activity can be blocked by incubation with levamisole. However, neither intestinal nor placental alkaline phosphatase can be blocked with levamisole. Therefore, tissues of this origin should be stained with peroxidase detection systems. Background staining due to endogenous biotin can be blocked through an avidinbiotin blocking step prior to the primary antibody incubation step. 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. Overexposure with the protein blocking solution ("Blocking Solution") can result in decreasing signal intensity. Therefore, we recommend washing away the Blocking Solution instead of just draining it away as in other procedures. A higher sensitivity can be obtained when a second chromogenic substrate step is used (2 x Fast Red, Permanent AP Red or New Fuchsin).

APPLICATIONS:

This 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 mouse, rabbit, rat, and guinea pig. 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:

8 ml **Blocking Solution Reagent 1** (RTU)

8 ml **Biotinylated Secondary Antibody, Polyvalent Reagent 2** (RTU)

8 ml **Streptavidin-AP-Conjugate Reagent 3** (RTU)

8 x 5 ml **Naphthol-Phosphate Buffer** (Substrate Buffer)

8 uds **Fast Red Tablets** (Chromogen)

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 or secondary antibody is minimized via incubation with a protein blocking solution ("Blocking Solution" provided with the 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 biotinylated secondary antibody is applied and incubated. This secondary antibody functions as a link between primary antibody and streptavidin-alkaline phosphatase-conjugate ("Streptavidin-AP-Conjugate"). A second washing is followed by the application of this conjugate. It binds to biotin at the secondary antibody. Any excess of unbound streptavidin-AP-conjugate 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.
- Preparation of the chromogenic substrate working solution:
Add 1 tablet (Fast Red Tablet) to one bottle of substrate buffer (Naphthol-Phosphate Buffer) and mix until tablet is dissolved completely. This solution should be used directly after preparation.

Staining Procedure:

1. Blocking Solution (protein block, Reagent 1) (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 2 min.
 5. Biotinylated Secondary Antibody, polyvalent (Reagent 2, yellow) 10-15 min.
 6. Washing with wash buffer 3 x 2 min.
 7. Streptavidin-AP-Conjugate (Reagent 3, red) 10-15 min.
 8. Washing with wash buffer 3 x 2 min.
 9. Fast Red, Permanent AP Red, NBT/BCIP or New Fuchsin 10-20 min. (Controlling the colour intensity via light microscope is recommended.)
 10. Stopping the reaction with distilled H₂O when the desired colour intensity is attained.
 11. Counterstaining and blueing
 12. Mounting: aqueous with Fast Red, permanent with Permanent AP Red, NBT/BCIP or New Fuchsin.
- See our web site at www.genova-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



Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Test number



Manufacturer



See instruction for use



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2 of 2

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 primary antibodies, 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.

Antibodies, buffered solutions for antigen retrieval, enzyme treatments, others highly sensitive detection systems, and other auxiliary reagents are available from Genova 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. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user.

Genova 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 Genova 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 Genova 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, rat or guinea pig.
6. The antigen 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. Try a pre-treatment such as heat pre-treatment or enzyme digestion. If you used a pre-treatment 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.

Non-specific background staining or overstaining:

1. Incomplete deparaffinisation.
2. Excessive tissue adhesive on slides.
3. Insufficient washing especially after the incubation with the enzyme conjugate 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 metabolized by endogenous alkaline phosphatase in the tissue. This undesired activity can often be suppressed using levamisole (see also Limitations of the procedure).
9. Non-specific binding of the secondary antibody to endogenous biotin in the tissue section. Carry out an avidin-biotin block before incubation with the primary antibody.

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:

Genova 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.

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Catalog number



Batch code



In Vitro diagnostic medical device



Temperature limitation



Expiration date



Test number



Manufacturer



See instruction for use



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