Product datasheet **MON-APP102**

Monosan Plus (AP) Polymer Bulk Kit, 1 Kit (100 ml/1,000 tests)

Reagents

Instructions for use

Product name

Monosan Plus (AP) Polymer Bulk Kit, 1 Kit (100 ml/1,000 tests)

Intended Use

The Plus AP Polymer Kit is designed for the qualitative detection of antigens in fixed paraffin-embedded tissue sections, in frozen tissue sections, and in cytological samples. It is developed for use in combination with mono- and polyclonal primary antibodies and sera obtained from mice and rabbits. The kit can be used for examining tissues fixed in different solutions, e.g. formalin (neutrally buffered), B5, Bouin, ethanol, or HOPE.

Applications

IHC-P, IHC-Fr, IF

Summary and explanation

The purpose of the immunohistochemical staining is to make tissue and cell antigens visible. The Plus AP Polymer Kit is a highly sensitive detection kit intended for use in immunohistochemistry and immunocytochemistry. The enzyme polymer in this kit 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 colourising reagent which permits microscopical analysis.
The test system is suitable for the detection of mono- and polyclonal primary antibodies and sera obtained from mice and rabbits. In contrast to other detection techniques, which often use the streptavidin-biotin system the Plus AP Polymer Kit avoids the problem of background staining caused by endogenous biotin in the tissue.

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Principle of 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 APpolymer 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 Permanent AP Red leads to the formation of a magentared product of reaction at the place of the target antigen.

Reagents provided

100 ml Blocking Solution Reagent 1 (ready-to-use)
100 ml PostBlock Reagent 2 (ready-to-use)
100 ml AP-Polymer (Mouse/Rabbit) Reagent 3 (ready-to-use)
Materials required but not supplied
Positive und negative control tissue
Xylene or suitable substitutes
Ethanol, distilled H2O
Reagents for enzyme digestion or heat pre-treatment
Wash buffer PBS or TBS
PAP Pen
Primary antibody (user-defined)
Primary antibody diluent
Negative control reagent
Chromogenic substrate
Counter stain solution
Mounting medium
Cover slips

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Storage and handling

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. They should not be used after the expiry date. 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

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

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 5 min.
5. PostBlock (Reagent 2, yellow) 20 min.
6. Washing with wash buffer 3 x 5 min.
7. AP-polymer (Reagent 3, red) 30 min.
8. Washing with wash buffer 3 x 2 min.
9. Permanent AP Red 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: permanent or aqueous with Permanent AP Red

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.

Trouble shooting

If you observe unusual staining or other deviations from the expected results which could possibly be caused by the reagents, please contact our technical support.
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.
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 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).

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.

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

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 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 (i.e. POLHRP-125). 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 BlockingSolution instead of just draining it away as in other procedures. We will guarantee 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

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. Microbial contamination of the reagents must be avoided, since otherwise non-specific staining might appear. ProClin 300 and ProClin 950 used for stabilisation. 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. Omata M et al. Am J Clin Pathol 73: 626-632, 1980

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