Product datasheet **MON-APP107**

Monosan Plus (HRP) Polymer anti-mouse, 1.000 tests

Reagents

Instructions for use

Product name

Monosan Plus (HRP) Polymer anti-mouse, 1.000 tests

Intended Use

The Plus HRP Polymer anti-Mouse kit is designed for the qualitative detection of antigens in fixed paraffinembedded tissue sections, in frozen tissue sections, and in cytological samples. It was developed for use in combination with monoclonal primary antibodies and sera obtained from mice. The reagent 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 HRP Polymer anti-Mouse kit is a highly sensitive detection kit intended for use in immunohistochemistry and immunocytochemistry. The enzyme polymer consists of several molecules of secondary antibodies covalently bound to several molecules of horse radish 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 monoclonal primary antibodies and sera obtained from mice. In contrast to other detection techniques, which often use the streptavidin-biotin system the Plus HRP Polymer antiMouse 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. Endogenous peroxidase activity in the tissue may cause non-specific staining. This enzyme activity can be blocked by incubation with 3% H2O2-solution (peroxide block). Background staining caused by unspecific binding of the primary antibody or the secondary antibody in the HRPpolymer 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 HRP-polymer is applied and incubated. Any excess of unbound HRP-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 AEC leads to the formation of a red-brown product of reaction at the place of the target antigen. The chromogen DAB forms a dark brown precipitate.

Reagents provided

100 mL HRP-Polymer anti-Mouse (ready-to-use)
Substrate systems recommended: Permanent AEC kit, AEC single solution, AEC substrate kit, DAB substrate kit, DAB High contrast kit. Materials required but not supplied:
Positive und negative control tissue
Xylene or suitable substitutes
Ethanol, distilled H2O
3% H2O2 solution
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 solution should be stored at 2-8°C without further dilution. Please store the reagent in a dark place and do not freeze it. Under these conditions the solution is stable up to the expiry date. It 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

Reagent 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. Peroxide blocking (3 % H2O2 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 30 min. 8. Washing with wash buffer 3 x 2 min. 9. AEC or DAB (Controlling the colour intensity via light microscope is recommended.) 5-15 min. 10. Stopping the reaction with distilled H2O when the desired colour intensity is attained 11. Counterstaining and blueing 12. Mounting: aqueous with AEC, permanent with DAB or Permanent AEC

Expected results

During the reaction of the substrate with horse radish 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.

Trouble shooting

If you observe unusual staining or other deviations from the expected results which could possibly be caused by the reagents, please read these instructions carefully, 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 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 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 horse radish peroxidase in the tissue. Maybe the hydrogen peroxide solution used for blocking was inactivated

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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 peroxidase or pseudoperoxidase activity may cause non-specific staining. The enzyme activity is blocked by incubation with hydrogen peroxide solution. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems (Omata et al, 1980). 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 can result in decreasing signal intensity. Sanbio 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 circumstances shall Sanbio be liable for any damages arising out of the use of the reagent provided

Precautions

Use by qualified personnel only. Wear protective clothing to avoid eye, skin or mucous membrane contact with the reagents. In case of the reagent coming into contact with a sensitive area, wash the area with large amounts of water. Microbial contamination of the reagent must be avoided, since otherwise non-specific staining might appear. ProClin 950 is used for stabilisation. A Material safety data sheets (MSDS) is 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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