Product datasheet **MON-APP123**

Monosan Plus (HRP) Anti-Mouse (AEC) Kit, 1 Kit (8 ml/80 tests)

Reagents

Instructions for use

Product name

Monosan Plus (HRP) Anti-Mouse (AEC) Kit, 1 Kit (8 ml/80 tests)

Intended Use

The Plus HRP Kits, Mouse is based on the streptavidin-biotin system. It is designed for 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 monoclonal primary antibodies and sera obtained from mice. The Plus HRP Kit, Mouse 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 Kit, Mouse 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 horse radish peroxidase. Visualisation occurs via an enzyme-substrate reaction in the presence of a colourising reagent which permits microscopical analysis. The biotinylated secondary antibody in the Plus HRP Kit, Mouse binds to mouse primary antibodies. Therefore this kit can detect monoclonal primary antibodies and sera obtained from mice.

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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 or secondary antibody is minimized by incubation with a protein blocking solution ("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 biotinylated secondary antibody is applied and incubated. This secondary antibody functions as a link between primary antibody and the streptavidin-horse radish peroxidase-conjugate ("Streptavidin-HRP-Conjugate"). A second washing is followed by the application of this conjugate. It binds to the biotin at the secondary antibody. Any excess of unbound streptavidin-HRP-conjugate is thoroughly washed away after incubation. The addition of the chromogenic substrate starts the enzymatic reaction of the horse radish peroxidase which leads to colour precipitation where the primary antibody is bound. The colour can be observed via a light microscope. The chromogen used determines the colour. The chromogen AEC (included only in kit MON-APP123) leads to the formation of a red-brown product of reaction at the place of the target antigen. The chromogen DAB (included only in kit MON-APP124) forms a dark brown

Reagents provided

8 ml Peroxide Block (ready-to-use)
8 ml Blocking Solution Reagent 1 (ready-to-use)
8 ml Biotinylated Secondary Antibody, Mouse Reagent 2 (ready-to-use)
8 ml Streptavidin-HRP-Conjugate Reagent 3 (ready-to-use)
7 x 5 ml AEC Substrate Buffer
3 ml AEC Concentrate (Chromogen)
Substrate systems recommended (if not included in the kit): 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
Pink PAP Pen
Primary antibody (user-defined)
Primary antibody diluent
Negative control reagent
Chromogenic substrate

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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. • The tissue sections have to be completely covered with the different reagents in order to avoid drying out. • Preparation of the chromogenic substrate AEC working solution (with MON-APP123 only): Add 2 drops (100 µl) of AEC Concentrate to one bottle of AEC Substrate Buffer and mix thoroughly. • Preparation of the chromogenic substrate DAB working solution (with MON-

Procedure

1. Peroxide Block (3% H2O2 solution) 10 min. 2. Washing with wash buffer 1 x 2 min. 3. Blocking Solution (protein block, Reagent 1) (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 2 min. 7. Biotinylated Secondary Antibody, Mouse (Reagent 2, yellow) 10-15 min. 8. Washing with wash buffer 3 x 2 min. 9. Streptavidin-HRP-Conjugate (Reagent 3, red) 10-15 min. 10. Washing with wash buffer 3 x 2 min. 11. AEC or DAB (Controlling the colour intensity via light microscope is recommended.) 5-15 min. 12. Stopping the reaction with distilled H2O when the desired colour intensity is attained 13. Counterstaining and blueing 14. 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 pretreatment or enzyme digestion. If you used a pre-treatment it should be extended. 5. Primary antibody not from mouse. 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 pretreatment 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. Incubation times were too short or primary antibody concentration too low. 7. 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 metabolised by endogenous horse radish peroxidase. Maybe the hydrogen peroxide solution used for blocking was inactivated. 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.

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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 or the endogenous biotin content may cause non-specific staining. The enzyme activity can be blocked by incubation with 3% H2O2 solution. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems (Omata et al, 1980). 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. Sanbio guarantees that the product will meet all requirements

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 sodium azide (NaN3) are used for stabilisation. 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) 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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