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See the following pages for more information!

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Monosan® Fast (AP) One-Step Polymer anti-Mouse/Rabbit/Rat

REF / Cat. No.: **MON-APP207** **60 tests, 6 ml**
MON-APP208 **1000 tests, 100 ml**

Instructions for use

Intended use
Monosan® Fast (AP) One-Step Polymer anti-Mouse/Rabbit/Rat 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 monoclonal 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.
It is intended for in vitro diagnostic use.

Summary and Explanation
The purpose of the immunohistochemical staining is to make tissue and cell antigens visible.
Monosan® Fast (AP) One-Step 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 alkaline phosphatase (AP). Visualization occurs via an enzyme-substrate reaction in the presence of a colorizing reagent which permits microscopical analysis. The reagent is suitable for the detection of mono- and polyclonal primary antibodies and sera obtained from mouse. In contrast to other detection techniques, which often use the streptavidin-biotin system the Monosan® Fast (AP) One-Step Polymer anti-Mouse/Rabbit/Rat avoids the problem of background staining caused by endogenous biotin in the tissue.

Principle of the 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”). 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 AP One-Step polymer is applied and incubated. Any excess of unbound AP-polymer 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 AP Red leads to the formation of a magenta-red product of reaction at the place of the target antigen. Other suitable chromogens are Fast Red (magenta-red), New Fuchsin (magenta-red) or NBT (blue-black) with its substrate BCIP.

Substrate systems recommended (if not included in the kit):

Permanent AP Red Kit	*Cat. No. MON-APP185*	*1250 tests*
	Cat. No. MON-APP186	*5000 tests*
Fast Red Substrate Kit	*Cat. No. MON-APP179*	*550 tests*
	Cat. No. MON-APP180	*1250 tests*
	Cat. No. MON-APP181	*5000 tests*
New Fuchsin Kit	*Cat. No. MON-APP182*	*60 tests*
	Cat. No. MON-APP183	*1250 tests*

Materials required but not supplied

- Positive und negative control tissue
- Xylene or suitable substitutes
- Ethanol, distilled H_2O
- Reagents for enzyme digestion or heat pre-treatment
- Wash buffer PBS or TBS (Cat. No. MON-APP166)
- PAP Pen (Cat. No. MON-APP190)
- Primary antibody (user-defined)
- Primary antibody diluent (Cat. No. MON-APP149)
- Negative control reagent
- Chromogenic substrate
- Counter stain solution
- Mounting medium
- Cover slips

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 Monosans' technical support or your local distributor.

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 (NaN_3), 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.

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. Blocking Solution (protein block, 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. AP One-Step polymer anti Mouse?Rabbit/Rat	**30 min.**
6. Washing with wash buffer	**3 x 2 min.**
7. Fast Red, Permanent AP Red, NBT/BCIP or New Fuchsin	**5-15 min.**

(*Controlling the colour intensity via light microscope is recommended*.)
8. Stopping the reaction with distilled H2O when the desired colour intensity is attained
9. Counterstaining and blueing
10. Mounting: aqueous with Fast Red, permanent with Permanent AP Red, NBT/BCIP or New Fuchsin

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.

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.

Limitations of the 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. MON-APP103).

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.

Monosan® guarantees 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 Monosan® be liable for any damages arising out of the use of the reagent provided.

Troubleshooting
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 Monosans’ technical support 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 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).

Performance characteristics

Monosan® has conducted studies to evaluate the performance of the kit reagents. The product has been found to be suitable for the intended use.

FOR RESEARCH USE ONLY, NOT FOR DRUG, DIAGNOSTIC OR OTHER USE.

Explanation of the symbols on the product label:

REF	Bestellnummer Catalog Number Reference du catalogue	LOT	Chargenbezeichnung Batch Code Code du lot		Reizend Irritant Irritant
	Gesundheitsschädlich Harmful Nocif		Giftig Toxic Toxique		Hersteller / Manufacturer / Fabricant Monosan® Frontstraat 2c 5405 PB Uden The Netherlands Tel: (+31) 413 251115 Fax: (+31) 413 266605 info@monosan.com www.monosan.com
	Verwendbar bis Use By Utiliser jusque				
	Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation		Lagerungstemperatur Temperature Limitation Limites de température		