

ZytoChem Fast AP One-Step Polymer anti-Mouse/Rabbit

REF / Cat. No.: ZUC068-006 60 tests, 6 ml
ZUC068-100 1000 tests, 100 ml

Instructions for use

Intended use

ZytoChem Fast AP One-Step Polymer anti-Mouse/Rabbit is designed for the qualitative detection of antigens in fixed paraffin-embedded tissue sections, in frozen tissue sections, and in cytological samples. It was developed for use in combination with mono- and polyclonal primary antibodies and sera obtained from mice or rabbit. 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 research use only.

Summary and Explanation

The purpose of the immunohistochemical staining is to make tissue and cell antigens visible. ZytoChem Fast AP One-Step Polymer anti-Mouse/Rabbit 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). Visualisation occurs via an enzyme-substrate reaction in the presence of a colourising reagent which permits microscopic analysis. The test system is suitable for the detection of mono- and polyclonal primary antibodies and sera obtained from mice or rabbit. Cross-reactivity with primary antibodies from rat has been observed. In contrast to other detection techniques, which often use the streptavidin-biotin system the ZytoChem Fast AP One-Step Polymer anti-Mouse/Rabbit 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 One-Step Polymer 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 AP One-Step Polymer is applied and incubated. Any excess of unbound 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 Permanent AP Red leads to the formation of a magenta-red product of reaction at the place of the target antigen.

Reagents provided

REF / Cat. No. ZUC068-100
100 ml ZytoChem Fast AP One-Step Polymer anti-Mouse/Rabbit (ready-to-use)

REF / Cat. No. ZUC068-006
6 ml ZytoChem Fast AP One-Step Polymer anti-Mouse/Rabbit (ready-to-use)

Substrate systems recommended:

Permanent AP Red Kit	Cat. No. ZUC001-125	1250 tests
	Cat. No. ZUC001-500	5000 tests

Materials required but not supplied

Positive and negative control tissue
Xylene or suitable substitutes
Ethanol, distilled H₂O
Reagents for enzyme digestion or heat pre-treatment
Wash buffer PBS or TBS (Cat. No. ZUC020)
Blocking Solution (for protein blocking, optional)
Pink PAP Pen (Cat. No. ZUC064, optimal)
Primary antibody (user-defined)
Primary antibody diluent (Cat. No. ZUC025)
Negative control reagent
Chromogenic substrate
Counter stain solution
Mounting medium
Cover slips

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.

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 is used for stabilisation. A Material safety data sheet (MSDS) is available upon request.

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

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| 1. Blocking Solution (<i>This step is optional.</i>) | 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 | 30 Min. |
| 6. Washing with wash buffer | 3 x 2 min. |
| 7. Permanent AP Red,
(<i>Controlling the colour intensity via light microscope is recommended.</i>) | 10-20 min. |
| 8. Stopping the reaction with distilled H ₂ O when the desired colour intensity is attained | |
| 9. Counterstaining and blueing | |
| 10. Mounting: permanent or aqueous with Permanent AP Red Kit | |

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). 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.

Zytomed Systems 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 Zytomed System 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 Zytomed Systems' 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. 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 Blocking Solution 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).

Performance characteristics

Zytomed Systems 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 and Morales AR Ann N.Y. Acad Sci 420:134-139, 1983



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Explanations of the symbols on the product label

Symbols are used in accordance with ISO 15223-1. Further symbols on the product label might be:



GSH02: Flammable



GSH05: Caustic



GSH07: Attention / Warning



GSH08: Systemic health hazards

RUO

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