

ZytoChem Plus AP Kit, Rabbit

REF / Cat. No.:	AP008RED-RB	80 tests (Permanent Red included), 8 ml
	AP060-RB	600 tests, 60 ml
	AP125-RB	1250 tests, 125 ml
	AP500-RB	5000 tests, 500 ml

Instructions for use

Intended use

ZytoChem Plus AP Kit, Rabbit is based on the streptavidin-biotin system. It is designed for the 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 mono- and polyclonal primary antibodies and sera obtained from rabbit. The ZytoChem Plus AP Kit, Rabbit 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 Plus AP Kit, Rabbit is a highly sensitive detection kit intended for use in immunohistochemistry and immunocytochemistry. The method based on the streptavidin-biotin system which means that a biotinylated secondary antibody binds to several molecules of a conjugate composed of streptavidin and alkaline phosphatase. Visualisation occurs via an enzyme-substrate reaction in the presence of a colourising reagent which permits microscopical analysis. The biotinylated secondary antibody in ZytoChem Plus AP Kit, Rabbit binds to rabbit primary antibodies. Therefore this kit can detect mono- and polyclonal primary antibodies and sera obtained from rabbit.

Principle of the method

Paraffin-embedded tissue sections are first deparaffinised and rehydrated. Background staining caused by unspecific binding of the primary or secondary antibody is minimized by incubation with a protein blocking solution ("Blocking Solution" provided with the 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 biotinylated secondary antibody is applied and incubated. This antibody functions as a link between primary antibody and streptavidin-alkaline phosphatase-conjugate ("Streptavidin-AP-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-AP-conjugate 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 Red (included only in kit AP008RED-RB) leads to the formation of a magenta-red product of reaction at the place of the target antigen. Other suitable chromogens are Permanent AP Red (magenta-red) or NBT (blue-black) with its substrate BCIP.

Reagents provided

REF / Cat. No. AP008RED-RB		
8 ml	Blocking Solution	Reagent 1 (ready-to-use)
8 ml	Biotinylated Secondary Antibody, Rabbit	Reagent 2 (ready-to-use)
8 ml	Streptavidin-AP Conjugate	Reagent 3 (ready-to-use)
8 x 5 ml	Permanent Red Buffer (Substrate Buffer)	
2 ml	Permanent Red Concentrate (Chromogen)	

REF / Cat. No. AP060-RB

4 x 15 ml	Blocking Solution	Reagent 1	(ready-to-use)
4 x 15 ml	Biotinylated Secondary Antibody, Rabbit	Reagent 2	(ready-to-use)
4 x 15 ml	Streptavidin-AP Conjugate	Reagent 3	(ready-to-use)

REF / Cat. No. AP125-RB

125 ml	Blocking Solution	Reagent 1	(ready-to-use)
125 ml	Biotinylated Secondary Antibody, Rabbit	Reagent 2	(ready-to-use)
125 ml	Streptavidin-AP Conjugate	Reagent 3	(ready-to-use)

REF / Cat. No. AP500-RB

500 ml	Blocking Solution	Reagent 1	(ready-to-use)
500 ml	Biotinylated Secondary Antibody, Rabbit	Reagent 2	(ready-to-use)
500 ml	Streptavidin-AP Conjugate	Reagent 3	(ready-to-use)

Substrate systems recommended (if not included in the kit)

<i>Permanent AP Red Kit</i>	<i>Cat. No. ZUC001-125</i>	<i>1250 tests</i>
	<i>Cat. No. ZUC001-500</i>	<i>5000 tests</i>
<i>BCIP/NBT</i>	<i>Cat. No. K006</i>	<i>150 tests</i>

Materials required but not supplied

Positive and negative control tissue
 Xylene or suitable substitutes
 Ethanol, distilled H₂O
 Wash buffer (Cat. No. ZUC020)
 Reagents for enzyme digestion or heat pre-treatment
 PAP Pen (Cat. No. LP0001)
 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 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.

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. Microbiological 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) for the pure substances are 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.
- Preparation of the chromogenic substrate working solution (with AP008RED-RB only):
 Add 2 drops (60 µl) of Permanent Red Concentrate to one bottle of Permanent Red Buffer (Substrate Buffer) and mix. This solution should be used directly after preparation.

Staining procedure

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|---|-------------------|
| 1. Blocking Solution (protein block, Reagent 1) (<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 2 min. |

5. Biotinylated Secondary Antibody, Rabbit (Reagent 2, yellow)	10-15 min.
6. Washing with wash buffer	3 x 2 min.
7. Streptavidin-AP-Conjugate (Reagent 3, red)	10-15 min.
8. Washing with wash buffer	3 x 2 min.
9. Permanent Red substrate-chromogen solution (with AP008RED-RB)	5 min.
10. Wash with distilled H ₂ O	1 min.
11. Permanent Red substrate-chromogen solution (with AP008RED-RB)	5 min.
12. Wash with distilled water	3 x 1 min.
13. Counterstaining and blueing	
14. Mounting: aqueous or permanent after dehydration	

* The incubation times should be adjusted, when using other substrate-chromogen systems.

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 binding.

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 or the endogenous biotin content 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. Background staining due to endogenous biotin can be blocked through an avidin-biotin 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.

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:

- Reagents were not used in the proper order.
- Chromogenic substrate solution was too old.
- Bleaching because chromogen and mounting medium are incompatible.
- 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.
- Primary antibody not from rabbit, but from a different species.
- The antigen/epitope was not stable in the fixation and/or pre-treatment procedure used. Try another fixation or pre-treatment.

Weak staining:

- Inadequate fixation or overfixation.
- Incomplete deparaffinisation.
- 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.
- Excessive incubation with Blocking Solution or insufficient washing after this step.
- Too much wash buffer remains on the slides after washing, diluting the reagents applied in the next step.
- 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.
- Incubation times were too short or primary antibody concentration too low.
- Chromogenic substrate solution was too old.

Non-specific background staining or overstaining:

- Incomplete deparaffinisation.
- Excessive tissue adhesive on slides.
- Insufficient washing especially after the incubation with the enzyme conjugate or the chromogenic substrate solution. These washings are critical.

- Tissue was allowed to (partially) dry out with reagents on.
- Unspecific binding of the primary antibody. Please use the Blocking Solution provided with this kit or dilute the primary antibody in appropriate diluents.
- Incubation time of the primary antibody was too long or primary antibody concentration too high.
- 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.
- Incubation time of the chromogenic substrate solution was too long or reaction temperature too high (e.g. if temperature in the laboratory is high).
- 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

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 420:134-9, 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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