ZytoChem Plus HRP Kit, Mouse

REF / Cat. No.:		
	HRP008AEC-MS	**80 tests (AEC included), 8 ml**
	HRP008DAB-MS	**80 tests (DAB included), 8 ml**
	HRP060-MS	**600 tests, 60 ml**
	HRP125-MS	**1250 tests, 125 ml**
	HRP500-MS	**5000 tests, 500 ml**

Instructions for use

Intended use
ZytoChem 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 ZytoChem Plus HRP Kit, Mouse 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 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 (HRP). 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 HRP Kit, Mouse binds to mouse primary antibodies. Therefore this kit can detect monoclonal primary antibodies and sera obtained from mice.

Principle of the 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% H_2O_2-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 HRP008AEC-MS) 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 HRP008DAB-MS) forms a dark brown precipitate.

Reagents provided

REF / Cat. No. HRP008AEC-MS			
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)**		

REF / Cat. No. HRP008DAB-MS

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	**DAB Substrate Buffer**		
3 ml	**DAB Concentrate (Chromogen)**		

REF / Cat. No. HRP060-MS

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

REF / Cat. No. HRP125-MS

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

REF / Cat. No. HRP500-MS

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

Substrate systems recommended (if not included in the kit)

Permanent AEC Kit	*Cat. No. ZUC054-200*	*2000 tests*
AEC Single Solution	*Cat. No. ZUC037-008*	*80 tests*
	Cat. No. ZUC037-125	*1250 tests*
AEC Substrate Kit	*Cat. No. ZUC042-050*	*500 tests*
	Cat. No. ZUC042-500	*5000 tests*
DAB Substrate Kit	*Cat. No. DAB057*	*500 tests*
	Cat. No. DAB530	*5000 tests*
DAB High Contrast Kit	*Cat. No. DAB500 plus*	*500 tests*
	Cat. No. DAB5000 plus	*5000 tests*

Materials required but not supplied

Positive und negative control tissue
Xylene or suitable substitutes
Ethanol, distilled H_2O
Wash buffer (Cat. No. ZUC020)
Peroxide Block (Cat. No. ZUC019)
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 (NaN_3) 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.

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 HRP008AEC-MS 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 HRP008DAB-MS only):
  Add 4 drops (200 µl) of DAB Concentrate to one bottle of DAB Substrate Buffer and mix thoroughly.

Staining procedure

1.	Peroxide Block (3% H_2O_2 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 H_2O when the desired colour intensity is attained	
13.	Counterstaining and blueing	
14.	Mounting: aqueous with AEC, permanent with DAB or Permanent AEC	

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 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.

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 peroxidase or pseudoperoxidase activity or the endogenous biotin content may cause non-specific staining. The enzyme activity can be blocked by incubation with 3% H_2O_2 solution. 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 Systems 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 mouse.

- The antigen 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. Try a pre-treatment such as heat pre-treatment or enzyme digestion. If you used a pre-treatment 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.
- 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 horse radish peroxidase. Maybe the hydrogen peroxide solution used for blocking was inactivated.

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

www.zytomed-systems.de

Zytomed Systems GmbH • Anhaltinerstraße 16 • 14163 Berlin, Germany • Tel: (+49) 30-804 984 990

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 For Research Use Only