

BioSite Histo Plus AP Kit, Mouse



REF No	KDB-10020	80 tests (Fast Red Included, 8 ml)
	KDB-10023	600 tests, 60 ml
	KDB-10021	1250 tests, 125 ml
	KDB-10022	5000 tests, 500 ml

Instructions for use

Intended use

BioSite Histo Plus AP Kit, Mouse 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 monoclonal primary antibodies and sera obtained from mice. The BioSite Histo Plus AP 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 in vitro diagnostic use.

Summary and Explanation

The purpose of immunohistochemical staining is to make tissue and cell antigens visible. BioSite Histo Plus AP 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 alkaline phosphatase. Visualization occurs via an enzyme-substrate reaction in the presence of a colourising reagent which permits microscopical analysis. The biotinylated secondary antibody in the BioSite Histo Plus AP 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. 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 secondary antibody functions as a link between primary antibody and the 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 Fast Red (included only in kit KDB-10020) leads to a magenta-red product of reaction at the place of the target antigen. Other suitable chromogens are Permanent AP Red (magenta-red), Fast Red (magenta-red) or NBT (blue-black) with its substrate BCIP.

Explanation of the symbols on the product label:

REF	Catalog Number Bestellnummer Reference du catalogue	LOT	Batch Code Chargenbezeichnung Code du lot	Manufacturer Nordic BioSite AB Propellervägen 4A S-183 62 Täby Sweden Tel: +46 (0)8 5444 33 40 Fax: +46 (0)8 756 94 90 info@nordicbiosite.se www.nordicbiosite.se
Use By Verwendbar bis Utiliser jusque		IVD	In Vitro Diagnostic Medical Device In vitro Diagnostikum Dispositif médical de diagnostic in vitro	
Consult Instructions for use Gebrauchsanweisung beachten Consulter les instructions d'utilisation		Temperature Limitation Lagerungstemperatur Limites de température		

Reagents provided

REF	No	KDB-10020	
8 ml		Blocking Solution	Reagent 1 (ready to-use)
8 ml		Biotinylated Secondary Antibody, Mouse	Reagent 2 (ready to-use)
8 ml		Streptavidin-AP-Conjugate	Reagent 3 (ready to-use)
8 x 5 ml		Naphthol-Phosphate Buffer (Substrate Buffer)	
8		Fast red Tablets (Chromogen)	

REF	No	KDB-10023	
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-AP-Conjugate	Reagent 3 (ready to-use)

REF	No	KDB-10021	
125 ml		Blocking Solution	Reagent 1 (ready to-use)
125 ml		Biotinylated Secondary Antibody, Mouse	Reagent 2 (ready to-use)
125 ml		Streptavidin-AP-Conjugate	Reagent 3 (Ready to-use)

REF	No	KDB-10022	
500 ml		Blocking Solution	Reagent 1 (ready to-use)
500 ml		Biotinylated Secondary Antibody, Mouse	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>	No. BCB-20041	1250 tests
	No. BCB-20042	5000 tests
<i>Fast Red Substrate Kit</i>	No. BCB-20036	550 tests
	No. BCB-20037	1250 tests
	No. BCB-20038	5000tests
<i>NBT/BCIP</i>	No. K0066	150 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 PAP Pen (Cat. No. BSC-9C7F3A)
 Primary antibody (user-defined)
 Primary antibody diluent (Cat. No. BCB-20005, BCB-20006)
 Negative control reagent
 Chromogenic substrate
 Counter stain solution
 Mounting medium
 Cover slips

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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 Nordic Biosite 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 (NaN₃) are used for stabilization. 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.

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- 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-MS only):
- Add 1 tablet (Fast Red Tablet) to one bottle of substrate buffer (Naphthol-Phosphate Buffer) and mix until tablet is dissolved completely. This solution should be used directly after preparation.

Typical staining procedure

1. Blocking Solution (protein block, Reagent 1) (*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 2 min.**
5. Biotinylated Secondary Antibody, Mouse (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. Fast Red, Permanent AP Red, NBT/BCIP or New Fuchsin **10-20 min.**
(*Controlling the colour intensity via light microscope is recommended.*)
10. Stopping the reaction with distilled H₂O when the desired colour intensity is attained
11. Counterstaining and blueing
12. Mounting: aqueous with Fast Red, permanent with Permanent AP Red, NBT/BCIP or New Fuchsin

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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.

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. Therefore, tissues of this origin should be stained with peroxidase detection systems (i.e. HRP125-MS). 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. A higher sensitivity can be obtained when a second chromogenic substrate step is used (2 x Fast Red, Permanent AP Red or New Fuchsin).

Nordic Biosite 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 Nordic Biosite be liable for any damages arising out of the use of the reagent provided.

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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 Nordic Biosite 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, 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 overstraining:

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 alkaline phosphatase in the tissue. This undesired activity can often be suppressed using levamisole (see section Limitations of the Procedure).
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.

Performance characteristics

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

Bibliography

Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983

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