Diagnostic BioSystems

IHC Made Affordable

Instructions for Large Volume Universal Alkaline Phosphatase Immunostaining Kit (For Mouse Primary Antibodies)

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy. Clinical interpretation of staining results should be accompanied by histological studies with proper controls. Patients' clinical histories and other relevant diagnostic tests should be utilized by a qualified person (s) when evaluating and interpreting results.

This kit is designed to label specific primary antibodies immunohistochemically on tissue sections. The immunohistochemical protocol defined in this brochure is just a guideline. We encourage the individual laboratory to optimize its own protocol baed upon tissue fixation conditions, primary antibodies employed, and the user's experience. These reagents were tested and quality controlled using tissue sections. They can also be optimized for cell smears and cytospin preparations. Sufficient reagents are provided to run 500 to 1000 tests.

Principles of the Procedure
The high affinity of the non covalent interaction between biotin and streptavidin (1x1015) forms the basis for this immunostaining kit. It requires the formation of an irreversible and specific linkage between biological macromolecules. The immunohistochemical applications of the interaction between avidin and biotin were introduced by Bayer et al. (1979), who described techniques for generating active biotinyl compounds such as biotin-N-hydroxysuccinimide and biotin hydrazine and for conjugating them to various organic compounds, including immunoglobulins and alkaline phosphatase (ALP). Streptavidin (SA) is a tetrameric protein (mol. wt. 4x15,000), isolated from the actinobacterium Streptomyces avidinii (Chaiet & wolf, 1964). Streptavidin can bind to four molecules of biotin. Streptavidin gives superior results compared to avidin because its isoelectric point is closer to a neutral pH, where as avidin is positively charged at a physiological pH. Streptavidin does not carry any carbohydrate side chain, whereas avidin is composed of 70% carbohydrate. Because of this, SA does not have the tendency to bind non-specifically. Primary antibodies bind to the target antigens in the tissue sections and the conjugated second antibody binds specifically to these receptor antibodies. The biotin conjugated secondary antibody, in turn, is traced by a streptavidin conjugated enzyme and can be visualized by an appropriate substrate/chromogen.

Introduction
Immunohistochemical techniques are spreading rapidly and the practice of anatomic pathology has undergone a revolutionary change since the development of these procedures (Nadji & Morales, 1983). Because of their versatility and sensitivity and specificity, immunoperoxidase stains are invariably the best stains when (and if appropriate) antibodies are available. With the ever increasing number of antibodies against cellular antigens, immunoperoxidase techniques now provide a powerful tool to resolve a wide array of diagnostic pathology. All immunohistochemical techniques require the specific antibody employed to be so labeled that they can be easily seen when attached to cellular antigens. At the same time the sensitivity of immunoperoxidase techniques are central to wide variety of specific antigen localization. Alkaline phosphatase based kits are of special value for staining tissues that have high endogenous peroxidase activity. Alkaline phosphatase is unaffected by endogenous peroxidase and therefore results in cleaner background. Our kit is based on direct SA-ALP conjugate technology. The linker reagent is biotinylated anti-mouse, capable of labeling primary antibodies raised in mouse.

Reagents Supplied

Bottle 1	Linker Reagent: 110mL clear yellow solution of biotinylated anti-mouse immunoglobulins.
Bottle 2	Tracer Reagent: 110mL clear brick red solution of conjugated streptavidin-alkaline phosphatase.

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Precautions

i) The reagents in this kit contain sodium azide. Follow instruction provided by your local authorities for disposal. If disposed in the sink, flush the drain pipe to avoid a reaction of sodium azide in the plumbing system.
ii) Once you start the immunostaining process, don't let tissue sections dry because it can cause undesirable background and artifacts.
iii) Interpretation of the results will be the sole responsibility of the user.

Storage

All the reagents should be stored at 2-8°C. Do not freeze. Do not use beyond the expiration date stated on the label.

Material required but not provided

Some of the reagents and materials required for IHC are not provided. Pretreatment reagents, detection systems, control reagents and other ancillary reagents are available from Diagnostic BioSystems. Please refer to the Diagnostic BioSystems website at www.dbiosys.com

Specimen Collection and Preparation

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

The user is advised to validate the use of the products with their tissue specimens prepared and handled in accordance with their laboratory practices.

Reagents Preparation

The reagents in this kit are provided in a ready to use format.

Positive and Negative controls

Each immunostaining run should include a known positive and a negative control to assure the proper functioning of staining system and valid interpretation of the results.

Positive control

A tissue which is known to contain the desired antigen and has given positive staining.

Negative control

One of the following should be used as negative control:
i) Instead of primary antibody, use the normal nonimmune serum from the same species of animal in which the primary antibody was raised.
ii) Instead of the primary antibody, use the buffer in which the primary antibody was diluted.
iii) Use a tissue known not to contain the desired antigen.
iv) Absorb the primary antibody with the appropriate antigen and use it instead of primary antibody.

Staining Protocol

Step I Removal of paraffin wax: Deparaffinize tissue sections according to the established procedure in your lab and bring tissues to wash buffer.

Step II Endogenous Alkaline Phosphatase Blocking: This is an optional step and should be performed only if tissue is suspected to have high endogenous alkaline phosphatase activity. Apply enough drops of Alkaline Phosphastase Blocker to cover tissue. Incubate for 5-10 minutes at room temperature.

Step III Washing: Drain off excess reagent. Rinse with wash buffer three times for 1 minute each time. Drain off excess buffer and carefully wipe slide around the tissue to remove excess buffer from the glass leaving the tissue wet.

Immunostaining Protocol I

This protocol is recommended for optimally fixed tissues with abundant antigens in the tissue and for high affinity primary antibodies.

Step I Primary antibody: Apply enough drops of primary antibody to cover the tissue section. Incubate according to the manufacture's recommended conditions. Wash and wipe slides as described above.

Step II Linker reagent: Apply enough drops of linker reagent to cover the tissue section. Incubate for 10 minutes at room temperature. Wash slides as described previously.

Step III Tracer Reagent: Apply enough drops of tracer reagent to cover the tissue section. Incubate for 10 minutes at room temperature. Wash slides as described previously.

Step IV Substrate/Chromogen: Apply working chromogen solution for 5-10 minutes at room temperature for color development. For best results, look under the microscope for signal development. Once desired signal to noise ratio is achieved, stop the reaction by washing slides in wash buffer. Note development time and follow it during subsequent incubations.

Immunostaining Protocol II

This protocol is recommended for less than optimally fixed tissues with low antigenic density in the tissue and for low affinity primary antibodies.

Step I Primary antibody: Apply enough drops of primary antibody to cover the tissue section. Incubate according to the manufacture's recommended conditions. Wash slides as described previously.

Step II Linker reagent: Apply enough drops of linker reagent to cover the tissue section. Incubate for 20 minutes at room temperature. Wash slides as described previously.

Step III Tracer Reagent: Apply enough drops of tracer reagent to cover the tissue section. Incubate for 20 minutes at room temperature. Wash slides as described previously.

Step IV Substrate/Chromogen: Apply working chromogen solution for 5-10 minutes at room temperature for color development. For the best results, look under the microscope for the signal development. Once desired signal to noise ratio is achieved, stop the reaction by washing the slide in wash buffer. Note development time and follow it during subsequent incubations.

Step V Wash slides and counter stain with an appropriate counter stain. Mount and observe staining under the microscope.
Enhanced Staining
If additional signal is desired, treat tissue sections with Alkaline Phosphatase Enhancer (DBS catalog # K011) for 1 minute after Step III. Drain off excess enhancer and add substrate/chromogen solution without any wash.

References

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