Diagnostic BioSystems

IHC Made Affordable

Universal Alkaline Phosphatase Immunostaining Kit (For Mouse and Rabbit Primary Antibodies)

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
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 based upon tissue fixation conditions, primary antibodies employed, and the user's experience. These reagents were tested and quality controlled using tissue sections, however, they can also be optimized for cell smears and cytospin preparations. The linker reagent is a cocktail of biotinylated anti-mouse and anti-rabbit immunoglobulins, capable of labeling mouse and rabbit primary antibodies.

Principles of the Procedure
The high affinity of the non-covalent interaction between biotin and streptavidin ($1x10^{15}$) 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. 4 x 15,000), 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 neutral pH, whereas avidin is positively charged at physiological pH. Streptavidin does not carry any carbohydrate side chain, whereas avidin is composed of 70% of 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 conjugated secondary antibodies bind specifically to these receptor antibodies. A 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, sensitivity, and specificity, immunoperoxidase stains are invariably the best stains when 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 labeled that they can be easily seen when attached to cellular antigens. At the same time the sensitivity of the 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.

Reagents Supplied

Bottle 1	Alkaline Phoshatase Enhancer: 10mL clear solution. Used to increase the intensity of signal.
Bottle 2	Linker Reagent: 10mL clear yellow solution of biotinylated anti-mouse and anti-rabbit immunoglobulins.
Bottle 3	ALP Tracer Reagent: 10mL clear cherry red solution of conjugated streptavidin alkaline phosphatase.

Detector Reagents	PermaRed/AP Buffer 15ml

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PermaRed/AP Chromogen 1ml

Precautions

i) Several 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 staining and artifacts.
iii) Interpretation of the results will be the sole responsibility of the user.

Storage

All of the kit components 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

Except for the chromogen, all reagents are provided in ready to use form.

Preparation of Substrate/Chromogen

Aliquot 1mL of PermaRed/AP Substrate Buffer in a mixing bottle. Add one drop (~20µL) of PermaRed/AP Chromogen. Replace tip, mix, and allow solution to reach room temperature before using.

Note: The working chromogen-substrate solution should be prepared fresh and used within 20-30 minutes of preparation. Any solution not used during this period should be discarded.

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 in the past.

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) Use 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.

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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 for 1 minute after Step III. Drain off excess enhancer and add substrate/chromogen solution without any wash.

References

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