Product Manual

SAView™ (mouse/rabbit-HRP, AEC) IHC kit

Catalog #: ADI-950-121

For Mouse and Rabbit Primary Antibodies

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTENDED USE

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 qualified persons 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. Depending upon tissue fixation conditions, the primary antibody employed, and the user's experience, we encourage individual laboratory to optimize its own protocol. These reagents were tested and quality controlled using tissue sections, however, they can also be optimized for cell smears and cytospin preparations. Sufficient reagents are provided to run 50 to 100 tests.

TEST PRINCIPLE

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 horseradish peroxidase (HRP). 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 as compared to avidin because its isoelectric point is closer to a neutral pH, whereas avidin is positively charged at a physiological pH. Streptavidin does not carry any carbohydrate side chain, whereas avidin is composed of 70% of carbohydrate. Because of these reasons, SA does not have the tendency to bind non-specifically. Primary antibodies bind to target antigens in the tissue sections. The conjugated secondary antibody binds specifically to these receptor antibodies. Biotin-conjugated second antibody, in turn, is traced by a streptavidin-conjugated enzyme and can be visualized by an appropriate substrate.

INTRODUCTION

Immunoperoxidase 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 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. Various investigators (Petrusz et al., 1983; Nagle et al. 1983; Giorno et al., 1984) have shown that direct SA-HRP conjugate technique is 4 to 8 times more sensitive than the avidin-biotin complex described by Hsu et al. (1981). Our kit is based on direct SA-HRP conjugate technology. The linker reagent is a cocktail of biotinylated anti-mouse and anti-rabbit, capable of labeling primary antibodies raised in mouse and rabbit.

REAGENTS SUPPLIED

Bottle 1	Peroxidase reagent: 10mL clear solution of 3% hydrogen peroxide (H_2O_2) to block endogenous peroxidase activity.
Bottle 2	Linker Reagent: 10mL clear yellow solution of biotinylated anti-mouse and anti-rabbit immunoglobulins.
Bottle 3	Tracer Reagent: 10mL clear brick red solution of conjugated streptavidin horseradish peroxidase.

Detector Reagents:

- 1.5mL of AEC Substrate Buffer.
- 1.5mL of AEC Chromogen.
- 1.5mL of 3% solution of H_2O_2 (Substrate).

PRECAUTIONS

i) AEC has been classified as a suspected carcinogen and can cause skin irritation. Avoid contact with clothes and exposed skin. If accidentally contacted, flush immediately with tap water.

ii) Several of these reagents contain sodium azide. Follow instruction provided by local authorities for disposal. If disposed in the sink, flush the drain pipe to avoid a reaction of sodium azide with the plumbing system.

iii) Once the immunostaining process is started, do not let tissue sections dry because it can cause undesirable background and artifacts.

iv) 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.

OTHER MATERIALS NEEDED

- Xylene or dewaxing agents
- Absolute alcohol
- Distilled or de-ionized water
- Counterstain
- IHC wash buffer (Prod. no. ADI-950-235)
- HighDef IHC mount (Prod. no. ADI-950-261)
- Oven or incubator
- Staining jars
- Microscope slide
- Microscope
- Diamond pencil
- Absorbent pads
- Primary antibodies

SPECIMEN PREPARATION

The purpose of immunohistochemistry is to localize, identify and whenever possible quantitate cell and tissue constituents. Optimal fixation of the tissue is the key to good immunostaining. Successful immunostaining depends on accurate preservation of the epitome looked for in its structural context. We recommend users to read an excellent article on tissue preparation by Larsson (1993) to understand the need and importance of optimal tissue fixation.

REAGENT PREPARATION

The Peroxidase Reagent, Linker Reagent, and Tracer Reagent are provided in a ready-to-use format. Please refer below for instructions to prepare the working AEC Substrate/Chromogen solution.

Preparation of working AEC Substrate/Chromogen solution

i) Transfer 5mL of de ionized or distilled water in a test tube.
ii) Add 2 drops of AEC Substrate Buffer and mix.
iii) Add two drops of AEC Chromogen to the diluted buffer and mix.
iv) Add two drop of 3% H_2O_2 (Substrate) and mix.

This working solution is stable for 2 hours. Any solution not used during this period should be discarded.

Positive and Negative controls

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

Positive control:

A tissue which is know 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 non-immune 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 the tissue sections according to the established procedure in your lab and bring tissues to wash buffer.

Step II Peroxidase Blocking: Apply enough drops of Peroxidase Block to cover the tissue. Incubate for 5 minutes at room temperature. Use 0.3% H_2O_2 (dilute the Peroxidase Block 1:10) for the frozen tissue sections, cell smears, and cytospin preparations.

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. Leave 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 the 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: Apply enough drops of linker reagent to cover the tissue section. Incubate for 10 minute at room temperature. Wash and wipe slides as described previously.

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

Step IV AEC Substrate/Chromogen: Apply working AEC Substrate/Chromogen solution for 10-20 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 and wipe slides as described previously.

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

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

Step IV AEC Substrate/Chromogen: Apply working AEC Substrate/Chromogen solution for 5-15 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.

Step V Wash the slides and counter stain them with an appropriate counter stain. Mount and observe slides under the microscope.

REFERENCES

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NOTES

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com

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