4plus HRP Universal Detection

Detection Kit
Control Number: 901-HP504US-090617

Catalog Number: HP504 US
Description: 1000 Slides

Intended Use:
For In Vitro Diagnostic Use

Summary & Explanation:
Streptavidin is a protein that has similar binding properties to egg white avidin. It is isolated from streptomyces avidinii. Streptavidin has a molecular weight of 60 KD and has 4 subunits. Each subunit can bind one molecule of biotin. Biotin is a water-soluble vitamin. Streptavidin has an extremely high binding affinity (Kd=10-15) for biotin. It has proven useful in the detection of antigens coupled with biotinylated secondary antibodies.
There are several advantages when using streptavidin versus an avidin complex (ABC). In contrast to avidin, streptavidin is not glycosylated and is therefore uncharged at neutral pH (6.5 versus 10). This lowers nonspecific background staining. Streptavidin also lacks carbohydrate side chains that may be another cause of non-specific background. Another key advantage of streptavidin is the significant increase in sensitivity (probably due to less steric hindrance), thus facilitating an increase in overall binding capacity.
Finally, streptavidin-enzyme conjugates are much more stable than an ABC complex. The ABC complex must be freshly made 30 minutes prior to use and is stable only for a few days. In contrast, a streptavidin-conjugate can be stored for up to 1-2 years. The reagent comes in a ready-to-use format, thus saving time and potential mistakes.
BIOCARE'S 4plus™ detection system has been developed to provide a significant increase in staining sensitivity. The vast majority of primary antibodies can be diluted two-fold compared to other commercially available detection systems. 4plus™ Universal Detection system can be used with both mouse and rabbit primary antibodies. After labeling the antigen with a primary antibody, a universal, affinity-purified, biotinylated secondary antibody is added to bind to the primary antibody. Horseradish peroxidase (HRP) labeled-streptavidin is then added to bind to the biotinylated secondary antibody. A chromogen/substrate is then applied and reacts with a specific enzyme to produce an intense color signal. 4plus™ detection systems work well with paraffin-embedded tissues, frozen sections and cell preparations.

Supplied As:
1. 4+ Biotinylated Universal Goat Link (GU600H) 4x25ml
2. 4+ Streptavidin HRP Label (HP604H) 4x25ml

Materials and Reagents Needed But Not Provided:
Microscope slides, positively charged
Desert chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Decloaking chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS/PBS)
Pretreatment Reagents*
Enzyme Digestion*
Avidin-Biotin Blocking Kit*(Labeled Streptavidin Kits Only)
Peroxidase block*
Protein block*
Primary antibody*
Negative Control Reagents*
Chromogens*
Hematoxylin*
Bluing Reagent*
Mounting medium*

* BIOCARE MEDICAL PRODUCTS: Refer to a BIOCARE MEDICAL Catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations:

Deparaffinization:
Deparaffinize slides in Slide Brite or xylene. Hydrate slides in a series of graded alcohol to water.
Peroxide Block:
Block for 5 minutes with BIOCARE's PEROXIDAZED 1.
Pretreatment Solution/Protocol:
Please refer to the respective primary antibody datasheet for recommended pretreatment solution and protocol.
Avidin/Biotin Block (Optional, tissue dependent):
Incubate for 10-20 minutes at RT with BIOCARE's Avidin.
Incubate for 10-20 minutes at RT with BIOCARE's Biotin.
Protein Block (Optional):
Incubate for 10-15 minutes at RT with BIOCARE's Background Sniper.
Primary Antibody:
Please refer to the respective primary antibody datasheet for incubation time.
Link:
Incubate for 10 minutes at RT with 4+ Biotinylated Universal Goat Link.
Label:
Incubate for 10 minutes at RT with 4+ Streptavidin HRP Label.
Chromogen:
Incubate for 5 minutes at RT when using BIOCARE's DAB.
Counterstain:
Rinse with deionized water. Incubate for 30 to 60 seconds with Tacha's Automated Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute.

Technical Notes:

Use TBS or PBS for washing steps.
Control Slides
A positive control slide should be prepared from tissue known to contain the appropriate antigen. A negative control slide should be prepared from the same tissue block from the patient. Buffer, mouse or rabbit IgG fraction or primary antibody diluent can be substituted for the primary antibody.
Optional: Internal Processing Control
A tissue-processing control slide is prepared from the same tissue block as the patient specimen. A Vimentin antibody (Cat. No. CM048) can be used as an internal control to determine if the patient specimen is over-fixed. Vimentin will stain virtually all tissues. This Vimentin antibody is very sensitive to over-fixation. Excessive fixation may cause crosslinking that masks target antigens. If the Vimentin control is completely negative or very weak, it may be an indicator that the patient sample was over-fixed.
perhaps cause false negatives.

Limitations:

Specimen Preparation
Appropriate tissue fixation is required to obtain optimum performance and reliable interpretations. The following are commonly used fixatives: 10% neutral buffered formalin, B5, Zinc formalin, alcohol-based fixatives Zamboni's and Bouin's.
Cell smears prepared from body fluids should be a monolayer of cells. Multilayers of cells can trap staining reagents and interfere with the interpretation of the results. Smears should be fixed immediately after preparation. Fixation of frozen or cytospin sections can be accomplished with 100% acetone or methanol at 4ºC for 10 minutes.

Quality Control:

Refer NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on Tissue Controls.

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Precautions:

This product is not classified as hazardous. The preservative used in this reagent is Proclin 950 and the concentration is less than 0.25%. Overexposure to Proclin 950 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 950 in this product does not meet the OSHA criteria for a hazardous substance. Wear disposable gloves when handling reagents. Specimens, before and after fixation and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.
Consult OSHA, federal, state or local regulations for disposal of any toxic substances.
Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact BIOCARE's Technical Support at 1-800-542-2002.

Troubleshooting Guide:

No Staining
1. Critical reagent (such as primary antibody) omitted.
2. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Secondary antibody at too low of a concentration.
6. Primary antibody incubation period too short.
7. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining
1. Tissue is either over-fixed or under-fixed.
2. Incubation time of incorrectly processed-the primary antibody too short
3. Low expression of antigen
4. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
5. Over-development of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.
8. Incorrect procedure in reagent preparation.
9. Improper procedure in test steps.

Non-specific or High Background Staining
1. Variable fixation time.
2. Endogenous alkaline phosphatase (not blocked with levamisole).
3. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
4. Tissue may need a longer or a more specific protein block.
5. Substrate is overly-developed.
6. Tissue was inadequately rinsed.
7. Deparaffinization incomplete.
8. Tissue damage or necrotic.

Tissues Falling-Off
1. Slides were not positively charged
2. A slide adhesive was used in the waterbath
3. Tissue was not dried properly
4. Tissue contained too much fat

Specific staining too dark
1. Concentrated antibody not diluted out properly (being used at too high of a concentration).
2. Incubation of primary antibody, link or label too long.

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