Starr Trek Universal HRP Detection System

Sodium Azide and Thimerosal Free Detection Kit
901-STUHRP700-071017

BIOCARE MEDICAL

Catalog Number: **STUHRP700 H, L10**

Description: 25, 110 ml

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 kDa and has 4 subunits. Each subunit can bind one molecule of biotin. Biotin is a watersoluble 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 Starr Trek detection system has been developed to provide a significant increase in staining sensitivity. Starr Trek 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. Starr Trek detection systems work well with paraffin-embedded tissues, frozen sections and cell preparations.

Starr Trek universal detection systems can be used with BioGenex and Dako prediluted or concentrated antibodies at a significant cost reduction.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

Starr Trek HRP Universal Detection (25ML)

1. Trekkie Universal Link (STU700H) 1x25 ml
2. TrekAvidin-HRP (STHRP700H) 1x25ml
3. Betazoid DAB Chromogen (BDB900C) 1x1ml
4. Betazoid DAB Substrate Buffer (DS900H) 1x25ml
5. Background Sniper (BS966H) 1x25ml
6. Mixing Vial (VL103) 1ea.

Starr Trek HRP Universal Detection (110ML)

1. Trekkie Universal Link (STU700L10) 1x110 ml
2. TrekAvidin-HRP (STHRP700L10) 1x110ml
3. Betazoid DAB Chromogen (BDB900G5) 1x5ml
4. Betazoid DAB Buffer (DS900L10) 1x110ml
5. Background Sniper (BS966L10) 1x110ml
6. Mixing Vial (VL103) 1ea.

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*
Primary antibody*
Negative Control Reagents*
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.

Species Reactivity:

N/A

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.

Protocol Recommendations:

Tissue Preparation:

1. Cut tissue 4-5 microns thick and float on a water bath. Do not use adhesives in the water bath.
2. Place the tissue section on a positive-charged slide.
3. Drain excess water off the slides.
4. Dry tissues 30-60 minutes in a 37ºC oven; and then dry for 30 minutes at 60ºC.

Starr Trek HRP-DAB Staining Protocol:

1. Deparaffinize warmed tissues sections in 3 changes of Slide Brite for 3 minutes each.
2. Hydrate slides in a graded series of alcohol (100%, 95% and 70 %) to tap water. Rinse slides in deionized water (D.I.)
3. Apply 4 drops of Peroxidazed 1 (endogenous peroxidase blocker). Incubate for 5 minutes. Wash in tap water and rinse in DI water.
4. Optional Pretreatment: Heat designated slides for 40 minutes at 95°C in Diva or Biocare's Borg Decloaker (or other HIER buffers). Cool slides for 20 minutes. Wash in tap water and rinse with D. I. Water. Alternatively, a pressure cooker can be used at 125°C for 30 seconds to 3 minutes (Biocare's Decloaking Chamber).
5. Optional Pretreatment: Digest designated tissues with either Carezyme I (Trypsin) for 10-20 minutes at 37ºC or Carezyme II (Pepsin) for 5 minutes at 37ºC. Wash in slides in tap water and rinse in D. I. water.
6. Carefully wipe around the tissue section to remove excess water. Make a hydrophobic barrier above and below tissue section with Super PAP Pen (approximately 30mm or 48mm in length). Do not allow tissues to dry out!
7. Flood all slides with 1X PBS wash buffer.

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8. Drain slides and apply 4 drops of Biocare's Background Sniper (protein blocker) for 15 minutes at room temperature.
9. Drain protein blocker and apply 4 drops of the appropriate Primary Antibody for 30-60 minutes. Apply 4 drops of antibody diluent or negative control serum to the negative control.
10. Wash in 2 changes of 1X PBS wash buffer for 2 minutes each. Drain slides.
11. Apply 4 drops of the Trekkie Universal Link. Incubate for 20 minutes at room temperature.
12. Wash in 2 changes of 1X PBS wash buffer for 2 minutes each. Drain slides.
13. Apply 4 drops of TrekAvidin-HRP (Label). Incubate for 10 minutes at room temperature.
14. Wash in 2 changes 1X PBS wash buffer for 2 minutes each. Drain slides.
15. Apply 4 drops of the Betazoid DAB Chromogen solution. Develop 3-5 minutes at room temperature.

Directions:

Add 1 drop of DAB Chromogen to 1.0ml of substrate buffer and mix well.
16. Wash in D. I. water.
17. Add 4 drops of CAT Hematoxylin for 30-60 seconds. Wash in tap water.
18. Blue nuclei in 1X PBS wash buffer for 1 minute. Drain slides.
19. Wash in tap water and rinse in D.I. water.
20. Dehydrate in 3 changes of 100% alcohol and clear in 3 changes of xylene.
21. Mount and coverslip.

Technical Notes:

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. This may influence the staining results of other antibodies in the panel, and perhaps cause false negatives.

Protocol Notes:

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.

Performance Characteristics:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control

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

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. Primary antibody incubation time 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.

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7. Deparaffinization incomplete.
8. Tissue damaged 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.

Limitations & Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

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