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Super Sensitive™ Double Staining Polymer Detection System

Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail

A Biotin-Free Detection System

Doc. No. 932-QS400-60K Rev. No.: K
Release Date: 24-Aug-2020

Ready-To-Use (60 slides)

QS400-60KE Super Sensitive ™ Double Staining Polymer Detection Kit II / DAB & Red.

QS410-YIKE Super Sensitive ™ Double Staining Polymer Detection Kit II / DAB & Red / Large Volume

Ready-To-Use (500 slides)

For In Vitro Diagnostic Use

I. INTENDED USE

The BioGenex Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail represents state-of-art technology in the detection of antigen-antibody binding reactions, such as in immunohistochemical staining applications. This system has been designed to provide you with unsurpassed performance when recommended protocols are followed. Because of the sensitivity enhancement achievable with these reagents, the optimal dilutions and incubation times for primary antibody-cocktails will vary, in some cases dramatically, from those which you may be accustomed to.

Biogenex Double Stain Detection System provides a robust detection method for visualizing multiple antigens simultaneously in the same tissue sample. The double stain detection system contains alkaline phosphatase (AP) and horseradish peroxidase (HRP); it provides excellent sensitivity and saves time when used with simultaneous double staining. The system is biotin-free; background staining caused by endogenous biotin is completely eliminated.

The reagent in this kit can be used in conjunction with mouse andrabbit IgG primary antibody-cocktails supplied by BioGenex for the immunohistochemical staining of different antigens associated with tumors and infectious agents. BioGenex also provides Large volume of Ready-to-Use Polymers Cocktails,
For optimal results, please read and follow the instructions in this manual carefully. If you have any questions, please contact BioGenex Customer Service at Toll Free (USA ONLY) 1-(800)-421-4149.

II. PRINCIPLES

The demonstration of antigens in tissues and cells by immunostaining is a two-step process involving first, the binding of an antibody to the antigen of interest, and second, the detection and visualization of bound antibody by one of a variety of enzyme chromogenic systems. The choice of detection systems will dramatically impact the sensitivity, utility, and ease-of-use of the method.

The Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail is a novel detection system using a non-biotin polymeric technology that makes use of alkaline phosphatase (AP) for rabbit and horseradish peroxidase (HRP) for mouse. As the system is not based on the biotin-avidin system, problems associated with endogenous biotin are completely eliminated.

Tissues or cell preparations are frozen or fixed, sectioned, and attached to slides. The sections are then dewaxed if paraffin-embedded, treated with an antigen retrieval solution if required, blocked with a proteinaceous blocking solution and then incubated with a primary antibody-cocktail. The bound primary antibody is detected by the addition of secondary antibody conjugated with alkaline phosphate with Fast Red and horseradish peroxidase polymer with DAB substrate. When adequate color development is seen, the slides are washed in water to stop the reaction, counterstained, and covered with a mounting medium.

The conventional biotin-rich procedure makes use of the fact that avidin/streptavidin has a high affinity for biotin. One or two enzyme molecules are conjugated to streptavidin that binds to the biotinylated secondary antibody.

The present system is an improved one that achieves signal amplification and thereby an enhanced sensitivity by increasing the number of enzyme molecules which are conjugated to the secondary antibody.

In both the above cases the secondary antibody binds to primary antibody that is bound to the antigen of interest, ultimately leading to the enzymatic conversion of the substrate.

III. REAGENTS AND MATERIALS SUPPLIED

A. Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail Kits

The kit (QS400-60KE) contains the following reagents and materials:

Peroxide Block HK111-5KT: Two vials (6 ml) of 3% hydrogen peroxide in
Water. Use upto 200ul/slide.

Power Block™ HK083-5KT: Two vials (6 ml) of a highly effective universal protein blocking reagent. Contains casein and proprietary additives in PBS with 0.09% sodium azide. Use upto 200ul/slide.

Rabbit Negative Control HK408-06R: One vial (6 ml) of non-immune serum in PBS with 0.09% sodium azide. Use upto 100ul/slide.

Mouse Negative Control HK119-06M: One vial (6 ml) of non-immune serum or immunoglobulins in PBS with 0.09% sodium azide. Use upto 100ul/slide.

HK597-06K Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail: One vial (6 ml) of anti-mouse and anti- rabbit IgG labeled with enzyme polymer in phosphate buffered saline with stabilizers and proclin 300. Use upto 200ul/slide.

Liquid DAB chromogen HK124-7KT: One vial (4 ml) of the DAB (diaminobenzidine) chromogen which offers a great sensitivity as an HRP colorimetric chromogen. Use 2 drops or 80ul in 1ml of DAB buffer.

Stable DAB Substrate Buffer HK 520-10K: One vial (10 ml) of this component is only for use with DAB chromogen and comprises Tris buffer containing the peroxide and stabilizers. Use upto 100ul/slide.

Red Reagent A HK970-10K: Two vials (4 ml each) of Substrate for alkaline phosphatase enzyme. Use upto 100ul/slide
Red Reagent B HK971-10K: Two vials (4 ml each) of Substrate for alkaline phosphatase enzyme. Use upto 100ul/slide
Red Reagent C HK972-10K: Two vials (4 ml each) of Chromogen which produces a red reaction product in the presence of alkaline phosphatase enzyme. Use upto 100ul/slide
Red Buffer D HK973-10K: Two vials (10 ml each) of 100mM Tris-HCl buffer with 1% Tween 20, pH 8.2-8.5. Use upto 100ul/slide

B. QS410-YIKE Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail **– Large Volume**

The kit (QS410-YIKE) contains the following reagents and materials:

HK597-50K Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail: One vial (50 ml). Use upto 100ul/slide

Note: It is recommended that the reagents may not be substituted across kit lot numbers.

IV. HANDLING, STORAGE AND SHELF LIFE

Precautions: This reagent kit is for laboratory use only. 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.

Use a safety pipetting device for all pipetting. Never pipet by mouth. Wear disposable gloves during staining procedures. Avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with plenty of water. Minimize microbial contamination of reagents or else an increase in non-specific staining may occur. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

Some reagents in this kit contain sodium azide as a preservative at concentrations of less than 0.1%. Sodium azide may be toxic if ingested and may be fatal if inhaled, swallowed, or absorbed through the skin. In case of exposure, obtain medical attention immediately. Sodium azide is not classified as a hazardous chemical at the concentration of these products. However, toxicity information regarding sodium azide at the product's concentration has not been thoroughly investigated. For more information, a Material Safety Data Sheet (MSDS) for sodium azide in pure form is available upon request. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal flush with large volumes of water to prevent azide build up in plumbing. (Center for Disease Control, 1976, National Institute for Occupational Safety and Health, 1976)[2,3].

Formaldehyde, 37% solution (formalin), used in specimen preparation, is harmful if inhaled, swallowed, or absorbed through the skin. Avoid inhalation, ingestion, or contact with the skin. It is classified as a potential carcinogen and may alter genetic material. Formalin is combustible. If contacted with eyes or skin, flush immediately with copious amounts of cold water.

DAB is classified as a possible carcinogen and can cause skin irritation upon contact. Avoid contact with skin. If contacted, flush immediately with copious amounts of water.

The user is urged to consult the MSDS for this product for further information on product hazards, precautions, and waste disposal. Consult Federal, State or local regulations for disposal of any potential toxic components.

Storage Conditions: The reagents in this kit are to be stored at 2-8°C (36-46°F). If reagents are stored under any conditions other than those specified in the package insert, performance must be verified by the user.

Expiration: See product labels for expiration dates. Do not use after expiration date stamped on the vial. The performance of the reagents in this kit is backed by the BioGenex Total Quality Assurance policy.

V. REAGENTS AND MATERIALS NEEDED BUT NOT SUPPLIED

Primary Antibody Cocktails*

Positive Control Slide*

Diluent and Rinse Buffer*

Due to inhibitory effects of some preservatives and buffer systems on certain enzymes, care should be exercised in choosing diluents and rinse buffers.

Mounting media*

Absorbent wipes

Microscopic slides*

Coverslips for slides

Light Microscope with 10X and 40X final magnification.

Deionized water, reagent grade

Add 1mL of Brij-35 in 1000mL of Deionized water.

Optional Pretreatment Reagents* Depending upon the antigen and the extent of tissue fixation, tissues may require pretreatment by heating in Antigen Retrieval solutions.

*These products are available from BioGenex. Please refer to the BioGenex Catalog for details or contact BioGenex Customer Service at Toll Free (USA ONLY) 1-(800)-421-4149.

VI. PROCEDURES

A. PREPARATION OF CONTROL SLIDES

Each staining run should include both positive and two negative control slides (mouse and rabbit control) to confirm (1) that the staining system is working properly, (2) that positive or negative staining is specific, and (3) that the correct procedure has been followed.

Positive Control: The positive control slide should be prepared from tissue known to contain the antigen under study. Whenever possible, positive control slides should be fixed in the same manner as the test samples. Positive control slides are available from BioGenex.

Negative Control: The negative control slide should be prepared from the same tissue block as the specimen. Both mouse and Rabbit negative controls should be included in each staining run to verify that a reagent is staining according to its correct specificity. For details contact BioGenex Customer Service at Toll Free (USA ONLY) 1-(800) 421-4149.

B. DILUTION OF PRIMARY ANTIBODY

BioGenex Ready-to-Use antibody cocktail have been optimally diluted for use with these reagents and should not require further dilution. For user-supplied antibodies, dilution may be necessary to avoid overstaining.

C. PREPARATION OF SUBSTRATES AND CHROMOGEN (See Handling Precautions, Section IV.)

DAB Kits: DAB (3,3'-diaminobenzidine) forms a brownish end product that is insoluble in alcohol and, therefore, is suitable for permanent mounting. two drops (2 drops ~80µl) of DAB chromogen is mixed with 1 ml of substrate buffer.

Permanent Fast Red Kits: Permanent Fast Red gives a red reaction product with alkaline phosphatase enzyme and is suitable for permanent mounting. Add Permanent Fast Red A [HK970] and incubate at room temperature for 1 – 5 minutes. To this, add Permanent Fast Red B [HK971] and incubate at room temperature for 10 minutes. Always use equal volumes of HK970 and HK971. Do not apply the wash step between the substrate and chromogen steps.

D. TISSUE PREPARATION

Please consult the Carson[4] and Elias[5] references for details on preparing tissue sections for immunostaining, including protocols on embedding, deparaffinization, and rehydration.

E. PRETREATMENT

For some primary antibodies, routine tissue fixation in aldehyde-containing fixatives can have adverse effects on antigenicity. Overfixation can lower sensitivity leading to false-negative staining. Recovery of antigens in paraffin sections often can be accomplished by using Antigen Retrieval pretreatment or with proteolytic digestion. Always consult the primary antibody data sheet for recommended pretreatment information.

Antigen Retrieval Pretreatment:

The Antigen Retrieval pretreatment technique (U.S. Pat. Nos. 5,244,787 and 5,578,452 and their foreign equivalents) has been shown to increase staining intensity and reduce background staining of many important markers in formalin-fixed tissue. Although microwave heating is believed to be the primary factor in the recovery of antigenicity, the pH of the Antigen Retrieval solution is an important co-factor for some antigens [6-8].

BioGenex offers a variety of Antigen Retrieval solutions covering a wide pH range. To determine which solution is best for each antibody, please refer to the antibody data sheet or call BioGenex Customer Service at 1-(800) 421-4149.

F. STAINING PROCEDURE

The tissue sections should not be allowed to dry out at any point during the rehydration and staining procedures.

The following protocol is applicable to both the manual and automated experiments.
1. Peroxide Block:
Apply Peroxide Block to cover the specimen according to tissue size or autostaining slide parameters. Incubate for 5-10 minutes at room temperature. Drain and blot gently around the section.

2. Power Block™
Apply the Power Block to cover the section and incubate for 5 minutes at room temperature. Drain and blot gently (Note: do not wash the tissue section with washing buffer).
(Antibodies attach non-specifically to highly charged sites. This non-specific binding can be minimized by the use of a proteinaceous blocking reagent such as the Power Block).

3. Application of Primary Antibody-Cocktail:
NOTE: Ensure that the primary antibody-Cocktail is at the proper dilution. BioGenex ready-to-use Super Sensitive™ antibodies have been optimally diluted for use with these reagents and should not require further dilution.
The detection system must be matched to the species of the primary antibody-cocktial. Blot slides around sections. Add appropriate volume of Primary Antibody-cocktail to cover specimen according to tissue size or autostaining slide parameters. Likewise, add negative control serum to the negative control slide. Incubate the slides for the recommended time period and at the recommended temperatures. Rinse well with buffer twice for 5 minutes each.

4. Application of Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP **Reagent:**
Blot slides around the sections. Add appropriate volume of Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Reagent to cover the specimen according to tissue size or autostaining slide parameters. Incubate for 30 minutes at Room Temperature. Rinse thoroughly with Buffer at least twice for 5 minutes each.

5. Application of DAB Substrate Solution and Permanent Fast Red Substrate Solution:
Blot slides around the sections. Add appropriate volume of Substrate solutions to cover the specimen according to tissue size or autostaining slide parameters. Apply following procedures in the dark. For Red kit: add 2 drops of each Red Reagent A, Red Reagent B, and Red Reagent C into 5ml of Red Buffer D (HK973), mix well, and incubate with samples for 30 minute at room temperature. For DAB: add 2 drops **(80ul)** of DAB chromogen (HK124) into 1ml of DAB buffer (HK520) to make the solution. Incubate with samples for 10 minutes at room temperature or until acceptable color intensity has been reached. * 6-8 minutes if using Xmatrx automated system. Note: do not apply a wash step between the substrate and Chromogen steps. Rinse well with deionized water.
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6. Counterstaining Procedures:
Immerse the slides in a bath of Mayer's hematoxylin for 1 minute, depending on the strength of hematoxylin used. Rinse slides with tap water. (See Appendix, Section X).

7. Mounting Procedures:
Aqueous Mounting: While slides are still wet, mount coverslip using 1-2 drops of aqueous mounting medium available from BioGenex. (See Appendix, Section X).
Permanent Mounting: For a permanent record, slides can be mounted in a permanent mounting medium such as SuperMount® mounting medium. Tilt the slide to fully cover the tissue, place in a horizontal position and allow the coating to harden as recommended. No coverslip is necessary. (See Appendix, Section X).

VII. EXPECTED RESULTS

Proper use of this kit both manually and with the BioGenex Automated Staining Systems will result in intense, clear staining at the antigen sites in both the specimen and positive control. Staining of the negative control should first be noted and this information should be used to determine the amount of specific staining seen when examining the patient specimen. Any deviation from these expected results should cause the user to question the results and consult the troubleshooting guide for assistance. In addition, interpretation of the staining result is the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic product or procedure.

VIII. TROUBLESHOOTING

PROBLEMS AND POSSIBLE CAUSES
A. Overstaining:
1. Concentration of primary antibody is too high.
2. Incubation time of primary antibody too long.
3. Substrate incubation too long
4. Slides inadequately rinsed.

B. Weak staining on all slides:
1. Omission of recommended pretreatment: i.e, Antigen Retrieval pretreatment or proteolytic digestion.
2. Concentration of primary antibody too low.
3. Omission of Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Reagent.
4. Incubation time with primary antibody too short.
5. Substrate too old.
6. Too much rinse buffer left on slides causing excessive dilution of reagents.
7. Incompatible counterstain or mounting media which dissolves reaction product.
8. Incorrect deparaffinization of tissue.

C. No staining on any slide:
1. Omission of primary antibody and Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP .
2. Incorrect procedure: reagents used in the wrong order.
3. Incorrect preparation of substrate/chromogen solution.
4. Sodium azide present in Peroxidase label incubation or rinse solution.

D. Staining positive control slide only(test slide shows no signal):
1. No antigen present or level too low for detection by staining procedure. Increase incubation time with primary antibody.
2. Improper preparation of specimen tissue causing denaturation of antigen.
3. Specimen fixed for too long in formalin. Antigen masked by aldehyde cross-linking and increased hydrophobicity of tissue. May be possible to recover antigenicity with Antigen Retrieval pretreatment techniques or enzyme predigestion.
4. Immunoreactivity diminished or destroyed during tissue processing due to high temperature. (Do not expose tissue to temperature in excess of 60ºC.) For initial validation of immunohistochemistry, a set of controls should be run with the antibody and tissue to be tested. Negative controls should show no staining if the reaction is specific to the antigen.

E. Background:
1. Endogenous peroxidase. Requires peroxide block.
2. Nonspecific protein binding in tissue. Requires a protein block.
3. Inadequate rinsing.
4. Primary antibody too concentrated.
5. Incomplete deparaffinization.
6. Substrate incubation too long.
7. Tissue dried out during staining protocol.
8. Antigen diffusion prior to fixation-avoid delays in processing of tissue.
9. For formalin-fixed tissues, factors such as time, temperature and pH of fixation can cause antigens to be masked by aldehyde cross-linking and an increased hydrophobicity of tissue. This can lead to nonspecific binding. It may be possible to recover antigenicity with Antigen Retrieval pretreatment or to reduce background with a blocking reagent.
10. Impaired morphology or loss of cellular detail. Avoid excessive proteolytic digestion. Damaged tissue or necrotic areas of stained specimen should be ignored. Tissue sections wash off slide during incubation.

F. If tissue sections wash off slide, be sure slides are silanized or coated with polylysine or equivalent material. Remove additives from water bath during transfer of tissue sections to slides.

If you have questions regarding either the use of the reagents in this kit or the results obtained, contact BioGenex Customer Service at 1-(800) 421-4149.

IX. LIMITATIONS

Anti-Rabbit Polymer-AP and Anti-Mouse Polymer-HRP Detection Cocktail demonstrate antigens that survive tissue fixation, embedding and sectioning. Correct treatment of tissues prior to fixation and embedding, while less critical for BioGenex Reagents, is still important for obtaining optimal results. Inconsistent results may be due to variation in fixation and embedding methods employed by different laboratories, as well as from inherent variations in tissue. The results from immunostaining must be correlated with other laboratory findings and the relevant controls. An internal tissue processing control (e.g. vimentin) may be used to reveal errors in tissue processing. Use of BioGenex Antigen Retrieval pretreatment technique may permit recovery of antigenicity in formalin-fixed tissue. Please call BioGenex for more information on these products and their use in the standardization of immunostaining results.
The clinical interpretation of any positive staining or its absence should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive staining or its absence should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents and methods to interpret the stained preparation. Staining is to be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase. (Omata, et al. 1980)[9]
Normal/non-immune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.
False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g., liver, breast, brain, kidney) depending on the type of immunostain used. (Nadji & Morales, 1983)[9].

For In Vitro Diagnostic Use

X. APPENDIX: REAGENTS AVAILABLE

This section lists a selection of our most popular ancillary reagents and supplies. See the BioGenex Catalog for details and a complete listing of the reagents and sizes available.

The following reagents and Biological Stains are suitable for laboratory and research use unless otherwise specified.

A. Rinse Buffer
Phosphate Buffered Saline (PBS), pH 7.6 (HK091)

B. Diluents for Primary Antibodies
Common Antibody Diluent (HK156)
Enhanced Antibody Diluent (HK941)

C. Blocking Reagents
Power Block™ Universal Blocking Reagent (HK085)
Peroxide Block (HK111)

D. Counterstains
Hematoxylin, Mayer's (HK100)

E. Mounting Media
Aqueous Mounting Media (HK099)
SuperMount® Permanent Aqueous Mounting Medium (HK079)

F. Enzymes for Tissue Digestion
Pepsin (EK000)
Trypsin (EK001)
Protease XXIV (EK002)

G. Antigen Retrieval solutions*
*Please refer to BioGenex Catalog for details on Antigen Retrieval Solutions.

H. Other Ancillary Supplies
OptiPlus™ Positive-Charged Microscope Slides (XT002)
Barrier slides XT012, XT013, XT014

XI. REFERENCES

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2. Centers for Disease Control. Decontamination of Laboratory Sink Drains to Remove Azide Salts. Center for Disease Control Manual Guide -- Safety Management, No. CDC-22, Atlanta, Georgia. April 30, 1976.
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