CE
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Super Sensitive™ Double Staining Polymer Detection System

For In Vitro Diagnostic Use

Catalog No.	Description
QS200-60KE 60 slides	Super Sensitive™ Double Stain Polymer Detection Kit I (Anti-Rabbit Poly HRP + Anti-Mouse Poly AP)/ DAB & Red
QS200-YADE 100 slides	XViz™ Double Staining Detection Kit I (Anti-Rabbit Poly HRP + Anti-Mouse Poly AP)/ DAB & Red
QS210-YIKE 500 slides	Super Sensitive™ Double Stain Polymer Detection Kit I/Large Volume (Anti-Rabbit Poly HRP + Anti-Mouse Poly AP)
QS400-60KE 60 slides	Super Sensitive™ Double Stain Polymer Detection Kit II(Anti-Mouse Poly HRP + Anti-Rabbit Poly AP)/ DAB & Red
QS400-YADE 100 slides	XViz™ Double Staining Polymer Detection Kit II(Anti-Mouse Poly HRP + Anti-Rabbit Poly AP)/ DAB & Red
QS410-YIKE 500 slides	Super Sensitive™ Double Staining Polymer Detection Kit II/Large Volume(Anti-Mouse Poly HRP + Anti-Rabbit Poly AP)

Intended Use

The BioGenex Super Sensitive™ Double Staining Polymer Detection System 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 antibodies will vary, in some cases dramatically, from those which you may be accustomed to.

Principles of the Procedure

The demonstration of antigens in tissues and cells by immunostaining is a two-step process involving the binding of an antibody to the antigen of interest and the detection and visualization of bound antibody by an enzyme chromogenic system. The choice of detection systems affects its sensitivity, utility, and ease-of-use.

The Anti-Mouse Polymer-HRP/Anti-Rabbit Polymer-AP and the Anti-Rabbit Polymer-HRP/Anti-Mouse Polymer-AP Detection Cocktails are a novel detection system using a non-biotin polymeric technology that makes use of alkaline phosphatase (AP) for mouse and horseradish peroxidase (HRP) for rabbit and vise versa. As the system is not based on the biotin-avidin system, problems associated with endogenous biotin are 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 incubated with a primary antibody cocktail. The bound primary antibody is detected by the addition of secondary antibody conjugated with AP with Permanent Fast Red and HRP polymer with DAB substrate. When adequate color development is seen, the slides are washed in water to stop the reaction, counterstained, and mounted.

The present system achieves signal amplification and thereby enhanced sensitivity by increasing the number of enzyme molecules conjugated to the secondary antibody.

Storage and Handling

Store at 2-8°C. Do not use after the printed expiration date.

Preparation of Reagents

To prepare DAB working solution, add 1 drop (40ul) of DAB chromogen to each mL of DAB substrate buffer. Always use freshly prepared DAB working solution.

To prepare Red reagent, apply following procedures in the dark. Add 20ul of each Red Reagent A, Red Reagent B, and Red Reagent C into 1.25ml of Red Buffer D (HK973), mix well, and incubate with samples for 30 minute at room temperature on Xmatrx.

Staining Procedure

Step/Reagent	Incubation Time (min)*	No. of Washes/ Rinses*	No. of Cycles*
Baking	15	0	0
EZ-DeWax™	3	3	3
EZ-AR™ Solution	15-20$^{\alpha}$	3	1
Peroxide Block	10	3	1
Power Block™	10	0	1
Antibody	20-60†	3	1
Poly-HRP + Poly-AP	30	3	1
DAB	10	5	1
Red	30	4	1
Hematoxylin	3	5	1
Alcohol	0	1	0
XMount™	10	0	1

*These parameters may be modified by the user.
α The antigen retrieval is specific to an antibody. Kindly see the antibody datasheet for the exact protocol for antigen retrieval.
†The Antibody incubation time is specific to antibody. Kindly see the antibody datasheet for the exact incubation time.

Precautions

Specimens and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Avoid microbial contamination of reagents to minimize non-specific staining. Wear suitable Personal Protective Equipment. Never pipette reagents by mouth. Avoid contact of reagents and specimens with skin and mucous membranes. If reagents or specimens come into contact with sensitive area, wash with copious amounts of water.

Some reagents in this kit contain sodium azide at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at these concentrations, but proper

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handling protocols should be observed. DAB is classified as a possible carcinogen and can cause skin irritation upon contact. For more information on product hazards, precautions and waste disposal, Material Safety Data Sheets are available upon request. Dispose of unused reagents according to Local, State and Federal Regulations.

Reagents and Materials Needed but Not Supplied

This section lists our most popular ancillary reagents and supplies, including reagents contained in some but not all of these kits, as laid out in the Materials and Reagents Provided table on p3. See the BioGenex Catalog for details and a complete listing of the reagents and sizes available.

Cocktail Antibodies	• Please refer to the BioGenex Catalog for details.
Rinse Buffer	• Phosphate Buffered Saline (PBS), pH 7.6 (HK091)
Diluents for Primary Antibodies	• Common Antibody Diluent (HK156) • Enhanced Common Antibody Diluent (HK941)
Protein Blocks	• Peroxide Block (HK111) • Power Block (HK083)
Chromogen Solution Components	• Liquid DAB Chromogen (HK124) • Stable DAB Substrate Buffer (HK520) • Fast Red (for Aqueous Mounting) (HK182-5KE)
Antigen Retrieval Solutions	• Please refer to the BioGenex Catalog for details.
Counterstain	• Hematoxylin (HK100)
Mounting Media	• XMount Permanent Mounting Medium (HX035)
Other Ancillary Supplies	• OptiPlus™ Positive-Charged Microscope Slides (XT002) • Barrier slides XT012, XT013, XT014

Limitations

It is recommended that the reagents not be substituted across kit lot numbers. Interpretation of the staining result is solely the responsibility of the user. Experimental results should be confirmed by a medically-established diagnostic product or procedure. Evaluation must be performed by a qualified pathologist.

Improper tissue handling and processing prior to immunostaining can lead to inconsistent results. Variations in embedding and fixation or the nature of the tissue may lead to variations in results. Endogenous peroxidase activity or pseudo peroxidase activity in erythrocytes and tissue biotin may result in non-specific staining based on the detection system employed. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give a false positive with horseradish peroxidase systems.Improper counterstaining and mounting may compromise the interpretation of results. Normal/non-immuneserafromthesame animal source as secondaryantiseraused in blockingstepsmay cause false-negativeor-positive resultsdue to naturalorauto-antibodies.

Expected Results

Proper use of this kit 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.

References

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