BioGenex
Accelerating the pace of precision medicine

CE
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New & Improved Super Sensitive™ 1-step Polymer-HRP Detection System

Catalog No.	Description
QD600-60KEN 60 slides	Ready to use,New & improved Super Sensitive™ 1-step Polymer-HRP Detection System/DAB, **for Manual Use**
QD620-YIKEN 500 slides	Ready to use,New & improved Super Sensitive™ 1-step Polymer-HRP Detection System/DAB, **for Manual Use**
QD630-XAKEN 1000 slides	Ready to use,New & improved Super Sensitive™ 1-step Polymer-HRP Detection System/DAB, **for Manual Use**
QD610-YAXEN 200 slides	Ready to use,New & improved Super Sensitive™ 1-step Polymer-HRP Detection System/DAB, **for i6000®**
QD610-YADEN 200 slides	Ready to use,New & improved Super Sensitive™ 1-step Polymer-HRP Detection System/DAB, **for Xmatrx®**

Intended Use

For In Vitro Diagnostic Use. Super Sensitive™ Polymer-HRP Detection Systems are designed for the chromogenic detection of antigen-antibody binding reactions with mouse and/or rabbit IgG and IgM primary antibodies to achieve highly sensitive and specific immunohistochemical staining.

Summary and Explanation

Antigen detection by immunohistochemistry (IHC) is a two-step process wherein the primary antibody binds to specific epitopes of the antigen of interest and that binding is detected by a chromogen. BioGenex Super Sensitive™ 1-step Polymer-HRP Detection Systems use a non-streptavidin-biotin proprietary micropolymer-complex technology to minimize background staining wherein the Polymer-HRP reagent is bound to the primary antibody and visualized by diaminobenzidine (DAB).

Storage and Handling

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

Quality Control

Follow the staining protocol for the kit and primary antibody exactly. Use a consistent volume of reagent across all slides.

Limitations

It is recommended that 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.

Pretreatment

Routine tissue fixation can have adverse effects on antigenicity, leading to false-negative staining. Recovery of antigens can often be accomplished using Antigen Retrieval pretreatment or proteolytic digestion. Antigen Retrieval pretreatment increases staining intensity and reduces background staining. BioGenex offers Antigen Retrieval solutions covering a wide pH range, as that is an important factor for some antigens. **To determine which solution is best for each antibody, refer to the antibody datasheet.**

Staining Procedure

The following IHC protocol applies to all Super Sensitive™ 1-step Polymer-HRP Detection Systems. See the Quality Control section and the user manual for more details.

1. Do not allow tissue sections to dry out at any point during the rehydration and staining procedures.
2. Always use freshly prepared DAB working solution at a ratio of 1 drop (40 ul) of DAB chromogen per 1 ml of substrate buffer.

Step	Incubation	Rinse
Dewax	10 min at Room Temperature (RT)	Alcohol 2 min, DI water 5 min
Antigen Retrieval	See primary antibody datasheet	DI water x3
Peroxide Block	5 min at RT	PBS wash buffer x3
Power Block™	5 min at RT	N/A
Primary Antibody	See primary antibody datasheet	PBS wash buffer x3
Poly-HRP	30 min at RT	PBS wash buffer x3
Substrate Solution	5-10 min at RT	DI water 5 min
Counterstain	1 min at RT	DI water 5 min, alcohol 2 min
Mount and Coverslip	N/A	N/A

Note: For automation, please refer to the factory default protocol on instrument software.

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 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

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request. Dispose of unused reagents according to Local, State and Federal Regulations.

Troubleshooting

In all cases, check that the recommended staining protocol has been followed. If you have questions regarding the use of the reagents in this kit or the results obtained, contact BioGenex Technical Support Department at 1-800-421-4149 or support@biogenex.com, or contact your local distributor to report unusual staining.

A. Weak staining on all slides:
- Increase the primary antibody concentration, if possible.
- **Double the primary antibody incubation time.**
- Increase the primary antibody incubation temperature.
- Check that all reagents are within their expiration dates.
- Tap or dab off excess buffer left on slides after rinsing.
- Incompatible counterstain and mounting may dissolve the reaction product.
- Ensure tissue is correctly deparaffinized.

B. No staining on any slide:
- Ensure the substrate-chromogen solution is prepared correctly.
- Sodium azide may inhibit staining if present in too high concentrations in peroxidase labels or rinse solution.

C. Staining on the positive control slide only:
- The antigen may be present at too low a level for standard detection;double the primary antibody incubation time.
- Improper specimen preparation may denature the antigen.
- The specimen may be over-fixed in formalin; try Antigen Retrieval pretreatment techniques or enzyme predigestion.
- Immunoreactivity may be diminished or destroyed during tissue processing due to high temperature; do not expose tissue to temperature in excess of 60ºC.

D. Nonspecific background staining or overstaining:
- Lower the primary antibody concentration.
- Lower primary antibody incubation time or temperature.
- Lessen time of substrate incubation.
- Rinse slides thoroughly.
- Use peroxide block to counteract endogenous peroxidase.
- Use a protein block to counteract nonspecific protein binding. Ensure the tissue is correctly deparaffinized.
- Check that the tissue did not dry out during staining.
- Delays in tissue processing prior to fixation may cause antigen diffusion.
- The specimen may be over-fixed in formalin; try Antigen Retrieval pretreatment techniques or enzyme predigestion.
- Avoid excessive proteolytic digestion if impaired morphology or loss of cellular detail is observed.

E. 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.

Reagents Available but Not Supplied

This section lists our most popular ancillary reagents and supplies, including reagents contained in some but not all of these kits. See the BioGenex Catalog for details and a complete listing of the reagents and sizes available.

Primary Antibodies	• See 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)
Enzymes for Tissue Digestion	• Pepsin (EK000) • Trypsin (EK001) • Protease XXIV (EK002)
Antigen Retrieval	• See the BioGenex Catalog for details
Negative Controls	• Mouse Negative Control (HK119) • Rabbit Negative Control (HK408)
Counterstain	• Hematoxylin (HK100)
Mounting Media	• Aqueous Mounting Media (HK099) • SuperMount® Permanent Aqueous Mounting Medium (HK079) • XMount Permanent Mounting Medium (HX035)
Other Ancillary Supplies	• OptiPlus™ Positive-Charged Microscope Slides (XT002) • Barrier slides XT012, XT013, XT014

Bibliography

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4. Nadji M and Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. Lab Med 14:767-770, 1983.
5. Shi S-R, Key ME, and Kalra KL. Antigen Retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J. Histochem. Cytochem. 39:741-748, 1991.

	Temperature Limitation		Use By Date
	In Vitro Diagnostic Medical Device		Batch Code
	Non-Sterile		Representative in the European Community
	Consult Instructions for Use		Manufacturer