BioGenex
48810 Kato Road, Suite 100E & 200E,
Fremont, CA 94538

Tel : +1 (800) 421-4149,
Fax: +1 (510) 824-1490,
support@biogenex.com

EC REP Emergo Europe,
Prinsessegracht
20 2514 AP
The Hague,
The Netherlands

XViz™ Detection Kit for Xmatrx
Cat. No. QD550-YCDE

Immunohistochemistry Detection System

Doc. No. 932-QD550-YCDE, Rev. No.: N
Release Date: 25-May-2022

Ready-To-Use (200 slides)
(Prepackaged for use on BioGenex Xmatrx® Staining Systems)

***For In Vitro* Diagnostic Use**

I. INTENDED USE

The BioGenex XViz™ Polymer-HRP 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.

II. PRINCIPLES OF THE PROCEDURE

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 XViz™ Polymer-HRP Detection System is a novel detection system using a non-biotin polymeric technology that makes use of two major components: Super Enhancer™ and a Poly-HRP reagent. As the system is not based on the biotin-streptavidin interaction, 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. The bound primary antibody is detected by the addition of secondary antibody conjugated with horseradish peroxidase polymer and 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-based 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 over the conventional biotin-based 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 detection methodologies, i.e. biotin-based and Polymer HRP-based, 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

XViz™ Polymer-HRP Detection System for Xmatrx
QD550-YCDE contains the following:

Xviz™ Detection Kit

EZ AR™ 1 (HX031-YCDE) 16ml: One vial of EZ AR™ 1. Use upto 80ul/slide.
EZ AR™ 2 (HX032-YCDE) 16ml: One vial of EZ AR™ 2. Use upto 80ul/slide.

Peroxide Block (HX026-YADE) 48ml: Three vials of 3% hydrogen peroxide in water. Use upto 200ul/slide.

Power Block™ Reagent (HX083-YADE) 48ml: Three vials of a highly effective universal protein blocking reagent. Contains casein and proprietary additives in PBS with 0.09% sodium azide. Use upto 200ul/slide.

Super Enhancer™ Reagent (HX027-YCDE) 32ml: Two vial of the reagent that enhances the signal and is used after the primary antibody incubation. Preserved with ProClin™ 300. Use upto 140ul/slide.

Poly HRP Reagent (HX028-YCDE) 32ml: Two vial of anti-mouse and anti-rabbit IgG labeled with enzyme polymer in phosphate buffered saline with stabilizers and ProClin™ 300. Use upto 140/slide.

Stable DAB Substrate Buffer (HX029-50DE) 52ml: Four vials of this component, which is only for use with DAB chromogen and comprises Tris buffer containing the peroxide and stabilizers. Use upto 200ul/slide.

Counterstain (Mayer's Hematoxylin) HX030-YADE, 48ml: Three vials of counterstain. Use upto 200ul/slide.

Liquid DAB Chromogen (HX010-07XE) 4ml: 1 barcoded vial containing 4mL of DAB (diaminobenzidine) chromogen which offers a great sensitivity as an HRP colorimetric chromogen. The insoluble, permanent brown precipitate which is formed has a high-contrast in photographs. In addition, the sensitivity of DAB can be enhanced by carrying out the reaction in the presence of nickel or cobalt chloride and/or by examining slides by reflection interference microscopy (10x – 100x sensitivity). Use upto 40ul or 1 drop in 1 ml of Substrate.

Xmount (HX035-YCDE) 15ml: One vial of Xmount.

Mixing Vials (HX615-YADE): 4 Empty Barcoded vial for mixing DAB Buffer and DAB Chromogen.

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

IV. HANDLING, STORAGE AND SHELF LIFE

Precautions: 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 (see BioGenex Automated Systems Catalog for details).

V. REAGENTS AND MATERIALS NEEDED BUT NOT SUPPLIED

Primary Antibody*

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. Refer to Appendix, Section X for recommendations.

Negative Controls*.

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 negative control slides 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. Instead of using a primary antibody to the target antigen, use an antibody to an antigen (e.g. vimentin) that is present in most tissues and is sensitive to tissue processing. This control can be used to identify false negatives in overfixed tissue, indicating when additional procedures (e.g. Antigen Retrieval pretreatment) may be required. (See Pretreatment Section VI.E). For details contact BioGenex Customer Service at Toll Free (USA ONLY) 1-(800) 421-4149.

B. DILUTION OF PRIMARY ANTIBODY

BioGenex Ready-to-Use antibodies 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. If overstaining is evident, a further 1:2 to 1:5 dilution of the primary antibody is recommended. (See appendix, Section X, for recommended primary antibody diluents).

C. PREPARATION OF SUBSTRATES AND CHROMOGEN
(See Handling Precautions, Section IV.)
Add 40ul or 1 drop of DAB chromogen per ml of DAB buffer. Always use freshly prepared DAB working solution.

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 (800) 421-4149.

F. STAINING PROCEDURE

Please read the Operator's Manual for the Xmatrx® Staining System for instructions on operating the instrument.

Summary of the IHC Protocol on the Xmatrx® Staining System

Step	Reagent	Incubation Time (min)*	No. of Washes/ Rinses*	No. of Incubations*
1	**Baking**	15	0	0
2	**XDeWax™**	3	3	3
3	**EZ-AR™ Solution**	20-25[α]	3	1
4	**Peroxide Block**	10	2	1
5	**Power Block™**	10[β]	0	1
6	**Antibody**	20-60[†]	3	1
7	**Super Enhancer™**	20	3	1
8	**Poly-HRP**	30	3	1
9	**DAB Buffer**	10	2+3	1
10	**Hematoxylin**	3	2+3	1
11	**Alcohol (Clearmount)**	0	1	0
12	**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.
[β] Power Block is an optional step not required for all the antibodies.
[†]The Antibody incubation time is specific to antibody. Kindly see the antibody datasheet for the exact incubation time.

VII. 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.

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. Incubation temperature of primary antibody too high.
4. Substrate incubation too long
5. 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. Incubation time with primary antibody too short.
4. Incubation temperature with primary antibody too low.
5. Substrate too old.
6. Too much rinse buffer left on slides causing excessive dilution of reagents.
7. Incorrect deparaffinization of tissue.

C. No staining on any slide:
1. Incorrect preparation of substrate/chromogen solution.
2. 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. Nonspecific protein binding in tissue. Requires Power Block™.
2. Inadequate rinsing.
3. Primary antibody too concentrated.
4. Incomplete deparaffinization.
5. Substrate incubation too long.
6. Tissue dried out during staining protocol.
7. Antigen diffusion prior to fixation-avoid delays in processing of tissue.
8. 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 non-specific binding. It may be possible to recover antigenicity with Antigen Retrieval pretreatment or to reduce background with a blocking reagent.
9. Impaired morphology or loss of cellular detail. Avoid excessive proteolytic digestion. Damaged tissue or necrotic areas of stained specimen should be ignored.
10. Tissue sections wash off slide during incubation.
11. 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 Toll Free (USA ONLY) 1-(800) 421-4149.

IX. LIMITATIONS

The XViz™ Detection System demonstrates 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.

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)[10].

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
XWash IHC Wash Buffer pH 7.6 (HX020).

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

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

D. Other Ancillary Supplies
Barrier slides XT134-SL, XT134-CL
Cover Slips XT118-50X, XT118-YRK

XI. REFERENCES

1. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. Fourth Edition, April 1999. Web edition available at http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf
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.
3. U.S. Department of Health and Human Services (NIOSH), Rockville, MD. Explosive azide hazard, Publication No. 78-127, 1976.
4. Carson FL. Histopathology: A Self-Instructional Text. ASCP Press, Chicago, 1990.
5. Elias JM. Immunohistopathology: A Practical Approach to Diagnosis. ASCP Press, Chicago, 1990.
6. 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.
7. Tacha, DE. and Chen T. Modified antigen retrieval procedure: calibration technique for microwave ovens. *J. Histotechnol*. **17**:365, 1994.
8. Shi S-R, Gu J, Kalra KL, Chen T, Cote R.J., and Taylor C.R. Antigen Retrieval technique: a novel approach to immunohistochemistry on routinely processed tissue sections. *Cell Vision*. **2:**6-22, 1995.
9. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immuno histochemistry. *Am J Clin Pathol* **73**:626-632, 1980.
10. Nadji M and Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. *Lab Med* **14**:767-770, 1983.

SUGGESTED READING

Brozman M. Immunohistochemical analysis of formaldehyde- and trypsin- or pepsin-treated material. *Acta. Histochem*. **63**:251-260, 1978.
Banks PM. Diagnostic applications of an immunoperoxidase method in hematopathology. *J. Histochem. Cytochem*. **27(8):**1192-1194, 1979.
Falini B and Taylor CR. New developments in immunoperoxidase techniques and their applications. *Arch. Pathol. Lab. Med.* **107**:105-117, 1983.
Hsu SM., Raine L and Fanger H. A comparative study of the peroxidase-anti-peroxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am. J. Clin. Pathol*. **75(5):**734-738, 1981.
Hsu SM., Raine L and Fanger. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. o*J. Histochem*. Cytchem. **29(4):**577-580, 1981.
Mukai K. and Rosai J. Application of immunoperoxidase techniques in surgical pathology. In: Progress in Surgical Pathology. Fenoglio CM and Wolff M, eds., Masson Publishing USA, Inc., New York, p. 15-49, 1981.
Sternberger LA, Hardy Jr PH., Cuculis JJ and Meyer HG. The unlabeled antibody-enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-peroxidase) and its use in identification of spirochetes. *J. Histochem. Cytochem*. **18**:315-333, 1970.
Taylor CR. Immunoperoxidase technique: Practical and theoretical aspects. *Arch. Pathol. Lab. Med*. **102**:113-121, 1978.
Taylor CR and Kledzik G. Immunohistologic techniques in surgical pathology - A spectrum of "new" special stains. *Hum. Pathol*. **12(7):**590-596, 1981.
Taylor CR and Cote RJ. Immunomicroscopy: a diagnostic tool for the surgical pathologist. W.B. Saunders Company, Philadelphia, 1994.