BioGenex
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BGX AP IHC Detection kit for NanoVIP

Cat. No.	Description
QA910-YAX	Ready-To-Use, BGX AP IHC Detection system For NanoVIP, Open System (100 slides)

Intended Use

For In Vitro Diagnostic Use. The BioGenex AP IHC Detection system is 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.

Principles of the Procedure

The demonstration of antigens in tissues and cells by immunohistochemistry 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 BioGenex AP IHC Detection kit is a novel detection system using a non-biotin polymeric technology that makes use of two major components: Multilink and AP Label, which are bound to primary antibody and visualized by Fast Red. It achieves signal amplification and enhanced sensitivity by increasing the number of enzyme molecules conjugated to the secondary antibody.

Reagents and Materials Supplied

Do not substitute reagents across kit lot numbers.

Name	Catalog No.	Amount per Kit	Recommended Volume per Slide
EZ-AR 1	HX043-08XN	1 x 8.5 ml	80 µl
EZ-AR 2	HX045-08XN	1 x 8.5 ml	80 µl
Peroxide Block	HX047-08XN	3 x 8 ml	180 µl
Protein Block	HX112-08X	3 x 8 ml	180 µl
Multi-Link	HX340-08X	2 x 8 ml	140 µl
AP Label	HX331-08X	2 x 8 ml	140 µl
Fast Red Buffer	HX180-07X	4 x 7 ml	180 µl
Fast Red Chromogen	HX181-006X	1 x 0.6 ml	*
Counterstain (Mayer's Hematoxylin)	HX063-08XN	3 x 8 ml	180 ul
Mixing Vial	HX616-10X	2 vials	N/A

*See Preparation and Use of Substrate Solution

Staining Procedure

The following is the protocol to be followed with BGX AP IHC Detection kit.

Step	Reagent	Incubation Time (min)*	No. of Washes/ Rinses*	No of Incubations*
1	*Baking	15	0	0
2	XDeWax™	3	3	3
3	Antigen Retrieval	20-25$^{\alpha}$	3	1
4	Peroxide Block	10	2	1
5	Protein Block	10$^{\beta}$	NA	1
6	Primary Antibody	20-60$^{\gamma}$	3	1
7	Multi-Link	20	3	1
8	AP Label	20	3	1
9	Substrate Solution	30	2+3	1
10	Hematoxylin	3	2+3	1
11	Alcohol (Clearmount)	0	1	0

*These parameters may be modified by the user.
αThe antigen retrieval is specific to an antibody. Kindly see the antibody datasheet for the exact protocol for antigen retrieval.
βProtein Block is an optional step not required for all the antibodies.
γThe antibody incubation time is specific to antibody. Kindly see the antibody datasheet for the exact incubation time.

Mounting:

Aqueous Mounting: While slides are still wet, mount coverslip using 1-2 drops of Aqueous Mounting medium available from BioGenex (HK099).
Permanent Mounting: For a permanent record, slides can be mounted in a permanent mounting medium such as SuperMount® mounting medium (HK079). 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.

Storage and Handling

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

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.**

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Dilution of Primary Antibody

BioGenex Ready-to-Use antibodies have been optimized for use with the recommended BioGenex Detection System and should not require further dilution.

BioGenex concentrated antibodies must be diluted in accordance with the recommended protocol when used with the recommended BioGenex Detection System.

Preparation and Use of Substrate Solution

To Prepare Fast Red working solution, apply following in the dark. Add 20µl of Fast Red Chromogen in 1ml of Fast Red Buffer and mix well. Incubate the samples with the working solution for 30mins at room temperature. Working solution hould be used immediately after preparation.

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. 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.

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.

Limitations of the Procedure

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-immunesera 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.

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.

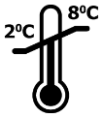
Reagents Available but Not Supplied

This section lists our most popular ancillary reagents and supplies. See the BioGenex Catalog for details and a complete listing of the reagents and sizes available. The following reagents are suitable for diagnostic histopathology, laboratory and research use unless otherwise specified.

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)
Enzymes for Tissue Digestion	• Pepsin (EK000) • Trypsin (EK001) • Protease XXIV (EK002)
Antigen Retrieval	• See the BioGenex Catalog for details
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

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. U.S. Department of Health and Human Services (NIOSH), Rockville, MD. Explosive azide hazard, Publication No. 78-127, 1976
3. Elias, J.M. Immunohistopathology: A Practical Approach to Diagnosis. ASCP Press, Chicago, 1990.
4. Shi, S-R, M.E. Key, and k.L. Kalra. 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.
5. Shi, S-R, J. Gu, K.L. Kalra, T. Chen, R.J. Cote, and C.R. Taylor. Antigen Retrieval technique: a novel approach to immunohistochemistry on routinely processed tissue sections. Cell Vision. 2:6-22, 1995.
6. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. Lab Med 1983; 14:767-770.

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