

## SUPER SENSITIVE™\* IHC DETECTION SYSTEMS

(LP000-ULE,LA000-ULE, QA900-9LE, QP900-9LE,  
QP300-XAKE)Doc. No. 932-IHCMAN-SS Rev. No.:K  
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### INSTRUCTION MANUAL

#### I. INTENDED USE

The BioGenex Super Sensitive™\* Detection Systems are intended for General purpose use for the chromogenic detection of antigen-antibody binding reactions in tissue specimens.

#### II. SUMMARY AND EXPLANATION

BioGenex Super Sensitive™ \* Link-Label HRP Detection System uses the streptavidin-biotin technology wherein the biotinylated secondary antibody which is bound to primary antibody reacts with enzyme labeled streptavidin and is then visualized by a chromogen. These detection systems may be used in immunohistochemical applications manually, or using BioGenex Automated Staining Systems.

#### III. PRINCIPLES OF PROCEDURE

Antigens in tissues and cells can be detected by a two-stage process: the binding of the primary antibody to specific epitopes and the subsequent detection of this binding by a colorimetric reaction. Tissue sections or cell monolayers are frozen or fixed, sectioned and attached to slides. The paraffin-embedded sections are then dewaxed. Some tissues, may need pretreatment with enzymes or heating in aqueous solution to unmask target antigens and allow binding of a specific monoclonal or polyclonal antibody. BioGenex antigen retrieval procedure consists of the microwave heating of formalin-fixed tissue in aqueous solution. It can recover almost full antigenicity with a vast majority of antibodies, including many that were previously unreactive with formalin-fixed tissue. The tissue sections are then treated with Power Block™ and Peroxide Block for blocking the non-specific protein-protein interactions and endogenous peroxidase, respectively. Levamisole can be used with alkaline phosphatase to block endogenous phosphatase staining.

**In the Link-Label system**, after the blocking steps the sections are incubated with a primary antibody specific for the antigen to be demonstrated. The sections are then incubated with biotinylated immunoglobulins (specific for various animal species) followed by incubation with the Label (an enzyme-labeled streptavidin). The streptavidin label has strong binding affinity to the biotin residues on the Multilink® antibody. The entire antibody-enzyme complex is then made visible by incubation with a chromogenic substrate until adequate color development is seen. Slides are washed in water to stop the reaction, counterstained and mounted. The type of chromogenic substrate depends upon the type of enzyme used, e.g. AEC and DAB can be used with Horseradish Peroxidase, Fast Red can be used with Alkaline Phosphatase.

**DAB** (diaminobenzidine) substrate offers the greatest sensitivity of all the Horseradish Peroxidase colorimetric chromogens. The insoluble, permanent brown precipitate has a high-contrast in photographs. In addition, the sensitivity 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 (10-100x sensitivity).

**AEC** (aminoethyl carbazole) is a colorimetric substrate for Horseradish Peroxidase. The bright reaction product is insoluble in water, but soluble in alcohol and xylene. The AEC substrate is suitable for immunohistochemistry (IHC), in situ hybridization (ISH), and membrane blotting applications. The AEC substrate is compatible with aqueous mounting media.

**Fast Red** (4-chloro-2-methyl-benzenediazonium salt) is a substrate for Alkaline Phosphatase and offers high sensitivity for light microscopic observations. The bright red dye precipitate produces maximal contrast with blue counterstains. The reaction product is insoluble in water, but soluble in organic solvents like alcohol and xylene. The dye

precipitate is inherently fluorescent and sections can be examined by fluorescence microscopy, which results in a 5x increase in signal-to-noise ratio.

#### IV. REAGENTS AND MATERIALS SUPPLIED

The following paragraph describes the composition of kit components. (For exact catalog number of kits and their contents please refer to Doc. No. 932-IHCMANE-Appendix).

#### Links

- **Ready-to-Use Links**  
**Multilink® (HK340), Mouse Link (HK335) and Rabbit Link (HK336):** Prediluted biotinylated anti-immunoglobulins in PBS with carrier protein and 0.09% sodium azide.
- **Concentrated Links**  
**Multilink® (HK268), Mouse Link (HK325) and Rabbit Link (HK326):** Concentrated biotinylated anti-immunoglobulins in PBS with carrier protein and 0.09% sodium azide.

#### Labels

- **Ready-to-Use Labels**  
**Alkaline Phosphatase Label (HK331):** Prediluted alkaline phosphatase conjugated streptavidin in PBS with carrier protein and 0.09% sodium azide.  
**Peroxidase Label (HK330):** Prediluted horse-radish peroxidase-conjugated streptavidin in PBS with carrier protein and 0.1% Proclin 300.
- **Concentrated Labels**  
**Alkaline Phosphatase Label (HK321):** Concentrated alkaline phosphatase conjugated streptavidin in PBS with carrier protein and 0.09% sodium azide.  
**Peroxidase Label (HK320):** Concentrated horse-radish peroxidase-conjugated streptavidin in PBS with carrier protein and 0.1% Proclin 300.

#### V. HANDLING, STORAGE AND SHELF LIFE

**Precautions:** This product is intended for in vitro diagnostic use. 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 (U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health, 1999).

Use a safety pipetting device for all pipetting. Never pipet by mouth. Wear disposable gloves during staining procedures. Avoid contact of reagents and specimens with the skin and mucous membranes. 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).

The user is urged to consult the MSDS of 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, they 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).

#### VI. REAGENTS AND MATERIALS NEEDED BUT NOT SUPPLIED

Some of the reagents and materials required for immunohistochemistry are not provided. Pretreatment reagents, primary antibody, control slides, control reagents and other ancillary reagents are available from BioGenex. Please refer to the product insert(s) of detection systems for detailed protocols and instructions on use of the reagents.

The immunohistochemistry procedure may need other lab equipment that is not provided including oven or incubator (capable of maintaining 56-60°C), BioGenex Automated Staining System, Humidity Chamber, Microwave oven, Staining Jars or baths, Timer (capable of 3-20 minute intervals), Wash Bottles, Absorbent Wipes, Microscopes slides (pre-

treated with poly-L-Lysine, BioGenex Catalog # XT002-SL), Coverslips, Lens paper and Light microscope with magnification of 200X.

\*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 or your local distributor.

#### VII. PROCEDURES

##### A. PREPARATION OF CONTROL SLIDES

Each staining run should include both positive and negative tissue control slides to confirm 1) that the staining system is working properly, 2) that positive and negative stainings are specific, and 3) that the correct procedure has been followed. If the positive and negative tissue controls fail to show proper staining the results of the test specimen should be considered invalid. Tissue controls should be fresh autopsy/biopsy/surgical specimens fixed, processed and embedded as soon as possible in the same manner as patient sample(s). Such a control monitors all steps of the analysis, from tissue preparation through staining. The use of a tissue section fixed or processed differently from the test specimen will act as a control for all reagents and method steps except fixation and tissue processing.

**Positive Tissue Control** (primary antibody on the known positive tissue): 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. One positive tissue control for each set of test conditions should be included in each run. Positive control slides are available from BioGenex. If the positive tissue controls fail to demonstrate positive staining, results with test specimens should be considered invalid.

**Non-specific Negative Reagent Control** (normal mouse IgG, serum, ascites or cell culture supernatant, or normal rabbit serum, or rabbit IgG versus the test specimen): Use of a non-specific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate non-specific staining and allow better interpretation of specific staining at the antigen site is recommended. Ideally, a negative reagent control contains the same source as primary antibody produced from the ascites or tissue culture supernatant in the same way as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix/solution as the primary antibody.

The negative reagent control slide should be prepared from the same tissue block as the test specimen. However, instead of using a primary antibody, use non-specific normal mouse IgG, ascites or tissue culture supernatant from the same source as the primary antibody (non-immune products are available from BioGenex for use as negative controls). The negative reagent control should be diluted with the same diluent to the same immunoglobulin or protein concentration as the diluted primary antibody. The incubation period for the negative reagent control should correspond to that of the primary antibody.

When panels of several antibodies are used on serial sections, the negatively staining areas of one slide may serve as negative/nonspecific binding background controls for other antibodies. To differentiate endogenous enzyme activity or nonspecific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate-chromogen or enzyme complexes (streptavidin) and substrate-chromogen.

**Negative Tissue Control** (primary antibody on the known negative tissue): Use a negative tissue control fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the IHC primary antibody for demonstration of the target antigen, and to provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can offer internal negative control sites to verify the IHC's performance specifications, but this should be verified by the user. This tissue should show absence of specific staining and provide an indication of specific background staining. It should also be used as an aid in interpretation of results. If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

**Tissue Processing Control** (an antibody targeting a widely-present antigen on the test specimen): The tissue processing 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 VII.E.) For details, contact BioGenex Customer Service at **(800) 421-4149** or your local distributor.

##### 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 Section XII, for recommended primary antibody diluents).

##### C. DILUTION OF CONCENTRATED LINK AND LABEL.

Dilute Concentrated Links and Labels 1:20 with the appropriate recommended diluent. Use HK165-XX for concentrated Link, HK156-XX for concentrated Alkaline Phosphate Label and HK157-XX for concentrated Peroxidase Label.

##### D. PREPARATION OF SUBSTRATES AND CHROMOGEN (See Handling Precautions, Section V.)

###### For AEC Kits:

**AEC Kits:** (3-amino-9-ethyl carbazole) forms a brick red end product that is soluble in organic solvent, do not use alcohol-containing solutions for dehydration and counterstaining. Counterstain and mount with aqueous-based mounting medium such as BioGenex Aqueous Mounting Medium (HK099-5K) or Super Mount Mounting Medium (HK079-5K, HK079-7K). One-step AEC (HK139) is a ready to use reagent.

**Prepare 1X Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) Substrate Buffer by diluting 5X H<sub>2</sub>O<sub>2</sub> Substrate Buffer 1:5 (HK171) in deionized water (add 1 ml 5X H<sub>2</sub>O<sub>2</sub> Substrate Buffer to 4 ml deionized water). Add 2 drops of AEC chromogen (HK121), as provided, to 5 ml of 1X H<sub>2</sub>O<sub>2</sub> Substrate Buffer. Mix well.**

**IMPORTANT:** Do not dilute the AEC Chromogen (HK121). Add as provided, to the 1X Substrate Buffer. The working solution of this AEC is stable for up to 5 hours at 20-26°C.

###### For DAB Kits Only:

**DAB Kits:** DAB (3,3'-diaminobenzidine) forms a brownish end product that is insoluble in alcohol and, therefore, is suitable for permanent mounting. Two drop(2 drops ~80µl) of DAB chromogen (HK124) is mixed with 1 ml of substrate buffer (HHK520). In case of automated kits add 20 drops of DAB chromogen (HK124-7K) to each optimizer vial of DAB substrate buffer (HK520-10X). This solution remains stable at room temperature (20-26°C) up to 6hours.

##### E. TISSUE PREPARATION

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

##### F. 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.

###### Enzyme Digestion:

For best results, optimize the enzyme digestion time according to the enzyme utilized, its concentration, the fixation time, tissue type, thickness of sections, and the specific antigen tested. For enzyme digestion kits, see Section XII.

###### Antigen retrieval Pretreatment:

The Antigen Retrieval pretreatment technique (U.S. Pat. Nos. 5,244,787 and 5,578,452) has been shown to increase staining intensity 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<sup>6-8</sup>.

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.

##### G. 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.

Step	Details
Application of Peroxide Block (optional) on tissue sample	<ul style="list-style-type: none"><li>• Cover tissue with reagent according to tissue size or autostaining parameters</li><li>• Incubation, <u>10</u> minutes, room temperature</li><li>• Rinse well with deionized water, then with buffer wash.</li></ul>

Notes: Required with HRP containing detection system only to quench endogeneous peroxidases.



Application of Power Block™ tissue sample

- Cover tissue with reagent according to tissue size or autostaining parameters
- Incubation, 5-10 minutes, room temperature
- Then drain and blot gently

Notes: Do not wash the tissue (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™).

Primary Antibody	<ul style="list-style-type: none"><li>Blot slides around sections. Cover tissue with reagent according to tissue size or autostaining parameters.</li><li>Likewise, add negative control serum to the negative control slide. Incubate the slides for the recommended time period and at the recommended temperatures.</li><li>Rinse well with buffer.</li></ul>
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Notes: Ensure that the primary antibody 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.

Link	<ul style="list-style-type: none"><li>Blot slides around the sections.</li><li>Cover tissue with reagent according to tissue size or autostaining parameters. Incubate, 20 minutes, room temperature.</li><li>Rinse well with buffer.</li></ul>
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Notes: The detection system must be matched to the species of the primary antibody.

Label	<ul style="list-style-type: none"><li>Blot slides around the sections.</li><li>Cover tissue with reagent according to tissue size or autostaining parameters. Incubate, 20 minutes, room temperature.</li><li>Rinse well with buffer at least three times.</li></ul>
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Substrate Solution	<ul style="list-style-type: none"><li>Blot slides around the sections.</li><li>Cover tissue with reagent according to tissue size or autostaining parameters. Incubate, 5-10 minutes, room temperature.</li><li>Rinse well with deionized water or buffer</li></ul>
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#### H. Counterstaining Procedures:

Immerse the slides in a bath of Mayer’s hematoxylin for 1-10 minutes, depending on the strength of hematoxylin used. Rinse slides with tap water. Optional: immerse in ammonia water for 10 seconds, then rinse with tap water. (See Section XII).

#### I. Mounting Procedures:

Aqueous Mounting: While slides are still wet, mount coverslip using 1-2 drops of aqueous mounting medium available from BioGenex. (See Section XII).

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 Section XII).

### VIII. EXPECTED RESULTS

Proper use of these kits, 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.

### IX. TROUBLESHOOTING

#### PROBLEMS AND POSSIBLE CAUSES

##### A. Overstaining:

- Concentration of primary antibody is too high.
- Incubation time of primary antibody too long.
- Incubation temperature of primary antibody too high.
- Substrate incubation too long
- Slides inadequately rinsed.

##### B. Green staining with peroxidase using AEC chromogen.

Indication of overstaining. Concentration of primary antibody too high.

##### C. Weak staining on all slides:

- Omission of recommended pretreatment: i.e, Antigen Retrieval pretreatment or proteolytic digestion.
- Concentration of primary antibody too low.
- Omission of Super Enhancer™ or Polymer-HRP Reagent.

- Incubation time with primary antibody too short.
- Incubation temperature with primary antibody too low.
- Substrate too old.
- Too much rinse buffer left on slides causing excessive dilution of reagents.
- Incompatible counterstain or mounting media which dissolves reaction product.
- Incorrect deparaffinization of tissue.

##### D. No staining on any slide:

- Omission of primary antibody, Polymer-HRP, and Super Enhancer™.
- Incorrect procedure: reagents used in the wrong order.
- Use of alcohol-based reagents (e.g. counterstaining, mounting media, buffers) after use of chromogens producing alcohol-soluble color products such as AEC.
- Incorrect preparation of substrate/chromogen solution.
- Sodium azide present in Peroxidase label incubation or rinse solution.

##### E. Staining positive control slide only (test slide shows no signal):

- No antigen present or level too low for detection by staining procedure. Increase incubation time with primary antibody.
- Improper preparation of specimen tissue causing denaturation of antigen.
- 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.
- Immunoreactivity diminished or destroyed during tissue processing due to high temperature. (Do not expose tissue to temperature in excess of 60°C.)
- Tissue block too old or epitope cut away.

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.

##### F. Background:

- Endogenous peroxidase. Requires peroxide block.
- Nonspecific protein binding in tissue. Requires a protein block.
- Inadequate rinsing.
- Primary antibody too concentrated.
- Incomplete deparaffinization.
- Substrate incubation too long.
- Tissue dried out during staining protocol.
- Antigen diffusion prior to fixation-avoid delays in processing of tissue.
- 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.
- Impaired morphology or loss of cellular detail. Avoid excessive proteolytic digestion. Damaged tissue or necrotic areas of stained specimen should be ignored.

##### G. 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. Make sure slides are adequately baked at 37°C for at least 30 minutes.

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

### X. LIMITATIONS

Super Sensitive™ \* Detection kits demonstrate antigens that survive tissue fixation, embedding and sectioning. Correct treatment of tissues prior to fixation and embedding, while less critical for BioGenex Super Sensitive™\* 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)

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)

### XI. PERFORMANCE CHARACTERISTICS

BioGenex has conducted studies to evaluate the performance of all its detection kits using several antibodies. The detection systems have been found to be sensitive and show specific binding to the primary antibody with minimal to no non-specific binding. BioGenex detection systems have shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated testing methods. BioGenex ensures product quality through 100% quality control for all products released and through surveillance programs.

### XII. 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 General Purpose Reagents and Biological Stains are suitable for diagnostic histopathology, 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 Common 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.

### XIII. REFERENCES

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Super Sensitive™\*, BioGenex®, Supermount®, MultiLink®, Power Block™, OptiPlus™ are trademarks of BioGenex.

For optimal results, please read and follow the instructions in this manual carefully. If you have any questions, contact BioGenex Customer Service at (800) 421-4149