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XISH™ One step polymer-HRP Detection System for Xmatrx® Cat. No. DF400-YADE

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Ready-To-Use (100 slides)
(Prepackaged for use on BioGenex Xmatrx® Staining System)

For In vitro diagnostics use

I. INTENDED USE

The XISH™ One step polymer-HRP ISH Detection System is intended for use in an automated *In Situ* Hybridization (ISH) procedure and is optimized for the detection of oligonucleotide probes. It is designed for the specific immunohistochemical detection of non-radioactive nucleic acid probes following hybridization to target DNA or mRNA sequences. Formalin-fixed, paraffin-embedded tissue sections are appropriate for use in this detection kit. This system has been designed to provide you with unsurpassed performance when recommended protocols are followed.

II. PRINCIPLES OF THE PROCEDURE

In Situ Hybridization (ISH) allows the detection and localization of definitive nucleic acid sequences within a cell or tissue. High specificity is ensured through the action of complementary nucleic acid binding sequences[1]. ISH techniques can be used to identify infectious agents in tissue sections[2-4], localize gene expression within individual cells[5-6], or detect specific DNA sequences in the genome of cells[7-9].

In an ISH procedure, fixed tissue sections are pretreated to expose target DNA or mRNA sequences. An appropriately labeled probe(in this case a fluorescein-labeled) is hybridized to the exposed target DNA or mRNA sequences in the tissue cells. Subsequent washing steps remove any probe that is non-specifically bound to the tissue section. Downstream detection of the labeled hybridized probe is done by the use of an anti-probe antibody. This includes incubating the slide with the sequential addition of mouse anti-probe primary antibody (in this case, anti-fluorescein antibody) and a Polymer-HRP-secondary antibody conjugate. After adding a substrate appropriate for the enzyme, a colored reaction product is precipitated at the location of the probe-target hybrid[10-15]. Microscopic examination of the slide provides a visual interpretation of the staining results.

The XISH™ One step polymer-HRP ISH Detection System is a novel detection system using a non-biotin polymeric technology that makes use of two major kit components: Super Enhancer™ and a Polymer-HRP reagent. As the system is not based on the biotin-avidin system, problems associated with endogenous biotin are completely eliminated.

The XISH™ One step polymer-HRP ISH Detection System is specifically designed for optimum immunohistochemical staining of paraffin-embedded tissue sections. The main advantages conferred by the system include:

1. Biotin-free detection system overcomes the background noise caused by endogenous biotin.
2. Very high specificity is inherent to the hapten labeled probe and nucleic acid interaction.
3. Very high sensitivity due to the Polymer-HRP Reagent used for detection.

III. REAGENTS AND MATERIALS SUPPLIED

XISH™ One step Polymer-HRP Detection ISH System for Xmatrx
DF400-YADE contains the following:

Liquid Pepsin (HK632-04XE) 5ml: Recommended for use with RNA targeting probes. BioGenex offers a variety of RNA targeting probes (Please refer to Catalog for details). For each slide/test use up to 50 µl.

Power Block (HX083-10XE) 10ml: One vial of Power Block. For each slide/test use up to 100 µl.

Nucleic Acid Retrieval (HX601-04XE) 5ml: NAR is recommended instead of Proteinase K when the kit is used with DNA targeting probes. For each slide/test use up to 50 µl

Peroxide Block (HX026-10XE) 10ml: One vial of 3% hydrogen peroxide in water. For each slide/test use up to 100 µl.

Liquid DAB chromogen (HX010-07XE) 7ml: One vial of the 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. Use upto 80ul or 2 drops in 1 ml of Substrate.

Stable DAB Substrate Buffer (HX029-05XE) 20ml: Four vials (5mlx4) of this component, which is only for use with DAB chromogen and comprises Tris buffer containing the peroxide and stabilizers. For each slide/test use up to 100 µl.

Polymer-HRP (HX943-04XE) 5ml: One vial of anti-mouse IgG labeled with enzyme polymer in phosphate-buffered saline with stabilizers and ProClin™ 300. For each slide/test use up to 50 µl.

Hematoxylin (HX030-10XE) 10ml: One vial of counterstain hematoxylin. For each slide/test use up to 100 µl.

Wash Solution A (HX839-10XE) 20ml: Two vials of sodium citrate saline (2X SSC). For each slide/test use up to 100 µl.

Wash Solution B (HX880-10XE) 20ml: Two vials of sodium citrate saline (0.5X SSC). For each slide/test use up to 100 µl.

Wash Solution E (HX946-10XE) 20ml: Two vials of sodium citrate saline (0.2X SSC). For each slide/test use up to 100 µl.

Wash Solution F (HX947-10XE) 20ml: Two vials of sodium citrate saline **(0.1X SSC).** For each slide/test use up to 100 µl.

Anti-Fluorescein Antibody (HX818-04XE) 5ml: One vial of Mouse anti-fluorescein antibody in PBS with carrier protein and 0.09% sodium azide. For each slide/test use up to 50 µl.

Hybridization Solution II (HX881-05KE) 6ml: One vial of Hybridization Solution II. For each slide/test use up to 50 µl.

Mixing Vials (HX615-YADE): 5 Empty barcoded vials 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: This reagent kit is for laboratory use only. 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

Probe*
Positive Control Slide*
Diluent and Rinse Buffer*
Due to the 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*.
Dehydration and Clearing Solution*
Mounting media*
Absorbent wipes
Microscopic slides*
Coverslips for slides
Light Microscope with 10X and 40X final magnification.
Deionized water, reagent grade

*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

NOTE: When the target to be detected is RNA, it is important to avoid contamination of the slides and reagents by ribonucleases (RNases - enzymes that degrade RNA) prior to and during hybridization. Be sure to wear gloves up to the hybridization step. All the reagents to be used up to the hybridization step are provided as RNase-free. Reagents to be prepared prior to use by users should also be prepared under RNase-free conditions.
(See handling precautions, Section IV.)

A. PREPARATION OF REAGENTS

DAB
DAB (3,3'-diaminobenzidine) forms a brownish end product that is insoluble in alcohol and, therefore, is suitable for permanent mounting.

- Two drops (80ul) of DAB chromogen is mixed with 1 ml of substrate buffer. Always use freshly prepared DAB working solution.

B. PREPARATION OF PROBES

This automated ISH detection system may be used to detect any appropriately fluorescein-labeled oligonucleotide probes. The optimal concentration of a probe depends on a number of parameters. Titration of a probe concentration is recommended. Lower concentrations may be employed to detect abundant nucleic acid species, while higher concentrations are required for rare ones.

C. TISSUE FIXATION

The automated ISH detection system is designed for use with routine formalin-fixed, paraffin-embedded tissue sections. For best results, specimens should be fixed in 10% neutral buffered formalin for 5 to 20 hours. Over-fixation may require prolonged incubation with Liquid Pepsin/NAR and may result in weak staining of positive tissue. Tissue processing conditions should be standardized in order to obtain consistent, reliable results. Use of a positive control probe is recommended to assess tissue processing.

D. PREPARATION FOR AUTOMATED ISH STAINING

The BioGenex automated ISH technology is a truly revolutionary technology. It converts a lengthy, cumbersome, labor-intensive, and irreproducible process into a fast, easy to perform and fully standardized procedure. The automated ISH is a high throughput system that needs minimal hands-on time and offers walk-away automation. The detection system contains all reagents necessary for staining up to 100 slides. The reagents are available in vials that can be directly loaded into reagent racks on a BioGenex Xmatrx® Staining System. Vials are Barcode labeled to permit rapid setup and on-board reagent inventory management.

Appropriately fluorescein-labeled probes are supplied by end-users. BioGenex also offers probes specifically designed for use in automation (Please refer to BioGenex catalog for details).

Please refer to the Operator's Manual of the BioGenex Xmatrx® Staining System being used for instructions on how to set up and run the instrument. Each laboratory is responsible for developing and validating optimal conditions for any specific probe and specific tissue sections chosen in a run. Liquid Pepsin or NAR treatment, denaturation, and chromogen development time may vary for different tissues.

E. STAINING PROCEDURE

Please read the Operator's Manual for the Xmatrx® Staining System for instructions on operating the instrument. Please see the next page for the staining procedure.

Summary of the ISH Protocol on the Xmatrx® Staining System

Ste p	Reagent	Incubation Time (min)*	No. of ISH/DI Rinses *	No. of Incubations*
1	**Baking**	15	0	0
2	**EZ-DeWax™**	3	5	3
3	**Alcohol**	6	2	2
4	**Liquid PepsinNAR[α]**	20/22	3-6	1
5	**Hybridization Solution II**	20	3	1
6	**Probe**	60-120	3	1
7	**Wash Solution[β]**	5	3	2
8	**Wash Solution[β]**	5	3	2
9	**Peroxide Block**	10	3	1
10	**Power Block**	5-10	0	1
11	**Anti Flourescein**	30-60	3	1
12	**Polymer HRP**	30-40	3	0
13	**DAB working solution**	10	3+2	1
14	**Hematoxylin**	1-3	3+2	1
15	**Clear Mount/ Alcohol**	-	1	0
16	**Xmount**	1	0	1

*These parameters may be modified by the user.

[α] The antigen retrieval is specific to a probe. Kindly see the probe datasheet for the exact protocol for antigen retrieval.

[β] Wash Solution (A/B/E/F) steps are specific to a probe. Kindly see the probe datasheet for the exact Wash solution to be used.

VII. EXPECTED RESULTS

Proper use of this detection kit will result in an intense stain at the specific site of the hybridized probe in positive test tissue with positive control probes. If staining is absent from any positive control slides, or present in any negative control slides, the test should be considered invalid. The interpretation of any test results is solely the responsibility of the user.

VIII. QUALITY CONTROL

Each *In Situ* Hybridization assay should include control slides to confirm that the detection kit is working properly and that the correct procedure has been followed.

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 probe:** The positive probe is known to be complementary to nucleic acid sequences in a test tissue slide that is processed in a manner identical to the slides that are being tested.
- **Negative control probe:** A negative probe is known not to be complementary to the target nucleic acid sequences that are being detected.

IX. LIMITATIONS

Correct treatment of tissues prior to and during fixation, embedding, and sectioning is important for obtaining optimal results. Inconsistent results may be due to variations in tissue processing, as well as inherent variations in tissue. The results from *In Situ* Hybridization must be correlated with other laboratory findings.

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™ ISH Wash Buffer, pH 7.6 (HX017-YIK).

B. ClearMount™

Dehydration and Clearing Solution, HX036-40D

XMount™
Permanent Mounting Medium, HX035-04D

C Other Ancillary Supplies

Barrier slides XT108-SL, XT108-CL

Cover Slips XT122-90X, XT122-YQK

XII. REFERENCES

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