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CE
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XISH™ One Step Polymer-HRP Detection System

Cat. No.	Description
DF400-25K	Ready to use Super Sensitive 1-Step Polymer HRP Detection Kit ISH(25 Test)
DF400-50KE	Ready to use Super Sensitive 1-Step Polymer HRP Detection Kit ISH (50 Test)
DF400-YADE	Ready to use XISHTM One Step Polymer HRP Detection System for Xmatrx (100 Test)

Intended Use

In Vitro Diagnostic Use. The Super Sensitive one-Step Polymer-HRP ISH Detection System is s 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 (FFPE) tissue sections are appropriate for use with this detection kit. This system has been designed to provide you with unsurpassed performance when recommended protocols are followed.

Principles of the Procedure

In Situ Hybridization (ISH) allows the detection and localization of definitive nucleic acid sequences within a cell or tissue. Complementary nucleic acid binding sequences ensure high specificity. ISH techniques can be used to identify infectious agents in tissue sections, localize gene expression within individual cells, or detect specific DNA sequences in a genome.

In an ISH procedure, fixed tissue sections are pretreated to expose target DNA or mRNA sequences. An appropriately labeled probe (in this case, with fluorescein) is hybridized to the exposed target sequences in the tissue. Subsequent washing steps remove any probe that is non-specifically bound. Anti-probe antibody detects labeled hybridized probe downstream by way of first a mouse anti-probe primary antibody (in this case, anti-fluorescein antibody) and then 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. Microscopic examination of the slide provides visual interpretation of the staining results.

This system is specifically designed for optimal immunohistochemical staining of paraffin-embedded tissue sections. The main advantages conferred by the system include less background noise due to biotin-free detection, high specificity inherent to the hapten labeled probe and nucleic acid interaction, and high sensitivity due to the Polymer-HRP Reagent used for detection.

Storage and Handling

Store at -20°C. Do not use after the printed expiration date.

Preparation of Reagents

This system may be used to detect any appropriately fluorescein-labeled oligonucleotide probe. The optimal concentration of a probe depends on a number of parameters. Titration of a probe concentration is recommended. Always use freshly prepared DAB working solution at a ratio of 1 drop (40 ul) of DAB chromogen per 1 ml of substrate buffer.

Reagents and Materials Supplied

Do not substitute reagents across kit lot numbers.

Name	Volume/Slide (25x25 Micr chamber)
Liquid Pepsin* (HK632)	≈100ul
NAR (HK/HX 601)	≈ 30-40ul
Peroxide Block (HK/HX 026)	≈100ul
Power Block (HK/HX 083)	≈100ul
Polymer-HRP (HK/HX 943)	≈100ul
DAB Buffer (HK520/HX029)	≈100ul
Hematoxylin (HK/HX 030)	≈100ul
Wash Solution A (HK/HX 839)	≈100ul
Wash Solution B (HK/HX 880)	≈100ul
Wash Solution E (HK/HX 946)	≈100ul
Wash Solution F (HK/HX 947)	≈100ul
Anti-Fluorescein Antibody (HK818/HX818)	≈80ul
Hybridization Solution (HK881/HX881)	≈80ul
DAB Chromogen (HK124/HX010)	≈ (1 Drop) 40ul/1ml buffer
Mixing Vial	NA

* Liquid pepsin should be kept at 37ºC for 30 minutes before use
Note- HK- for manual kit, HX- For automation

Staining Procedure

Reagent	Incubation Time (min)*	No. of ISH/ DI Rinses*	No. of Cycles*
Baking	15	0	0
EZ-Dewax™	3	5	3
Alcohol	6	2	2

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Liquid Pepsin / NAR**	20/22	3-6	1
Hybridization Solution II	20	3	1
Probe	60-120	3	1
Wash Solution***	5	3	2
Wash Solution***	5	3	2
Peroxide Block	10	3	1
Power Block	5-10	0	1
Anti-Fluorescein	30-60	3	1
Polymer HRP	30-40	3	0
DAB Working Solution	10	3+2	1
Hematoxylin	1-3	3+2	1
Clear Mount / Alcohol	-	1	0
XMount	1	0	1

*These parameters may be modified by the user.
**The antigen retrieval is specific to a probe. See the probe datasheet for the exact protocol for antigen retrieval.
***Wash Solution (A/B/E/F) steps are specific to a probe. See the probe datasheet for the exact wash solution to be used.

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.

Reagents and Materials Needed but Not Supplied

Product Name*	Description	Cat #
Micro ChamberSlides	25x25 mm	XT108-SL
		XT108-CL
Coverslips	25x25 mm	XT122-90X
		XT122-YQX
EZ-DeWax™	Deparafinizing reagent	HX015-XAK
Rinse Buffer	ISH Wash Buffer, pH 7.6	HX017-YIK
ClearMount™	Dehydration / Clearing Solution	HX036-40D
XMount™	Permanent Mounting Medium	HX035-04D
Probe/s	Assorted	Assorted

*These products are available from BioGenex. Please refer to the BioGenex Catalog for details or contact BioGenex Customer Service at 1-800-421-4149.
Also needed are a fluorescence microscope with appropriate filter set, deionized water, and reagent grade absolute ethanol.

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

Limitations

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 or pseudo peroxidase activity in erythrocytes may result in non-specific staining. 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.

References

1. Polak, J.M., et al. In Situ Hybridization: Principles and Practice. Oxford University Press, Oxford, 1990.
2. Margiotta M., et al. Comparison of three commercial kits for in situ detection of viral DNA. J. Histotech. 19(2):139-142, 1996.
3. Smith, K., et al. C-erbB-2 amplification in breast cancer: detection in formalin-fixed, paraffin-embedded tissue by In Situ Hybridization. Hum. Pathol. 25:413-418, 1994.
4. Hopman, A.H., et al. Detection of numerical chromosome aberrations using In Situ Hybridization in paraffin sections of routinely processed bladder cancers. Mod. Pathol. 4:503-513, 1991.

	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