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eFISH Histo
Cat. No. DF500-20XE

Doc. No: 932-DF500-20XE Rev. No. D
Release Date: 18-Oct-2021

Ready-To-Use (20 slides)
(Prepackaged for FISH use)

For In Vitro Diagnostic Use

I. INTENDED USE

eFISH Histo kit is intended for use in a Fluorescence *in Situ* Hybridization (FISH) procedure and it is optimized for the detection of target nucleic acid sequence. It is designed for the specific fluorescence detection of fluorescent labeled nucleic acid probes following hybridization to target DNA or RNA sequences. Formalin-fixed, paraffin-embedded (FFPE) 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

Fluorescence *in situ* hybridization (FISH) is a robust cytogenetic technique used for the detection of chromosomal aberrations, presence or absence of specific DNA sequence in native context. In this technique florescent probes bind to the target sequence of DNA chromosome. High specificity and sensitivity coupled with rapid, accurate result has proven the role of FISH in both research and diagnosis of solid tumor and hematological malignancies. As technique of cancer cytogenetics, FISH, can be used to identify genetic aberrations viz., deletions, amplification, duplication and translocation in tissue sections or within individual cells. FISH is also used for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets in cells, circulating tumor cells, and tissue samples[1,2,3,4,5].

In an FISH procedure, fixed tissue sections are pretreated to expose target DNA or mRNA sequences. An appropriately labeled probe is hybridized to the exposed target DNA or mRNA sequences in the cells. Subsequent stringent washing steps remove any probe that is non-specifically bound to the tissue section. Subsequently slides are mounted using DAPI/antifade and can be visualized under fluorescence microscope using appropriate filter set.

III. REAGENTS AND MATERIALS SUPPLIED

The BioGenex eFISH Histo kit is a novel system for processing formalin fixed paraffin embedded (FFPE) tissue. This kit contains reagents for pretreatment and post-hybridization stringency washings of the tissue. This kit is to be used for BioGenex eFISHiency FISH processing platforms Xmatrx Infinity/ELITE, Nano VIP, and Xmatrx Nano along with BioGenex eFISH probes. Also this kit can be used for manual procedure. eFISH probes can be ordered separately, a complete detail of eFISH probes is available on our company website.

BioGenex eFISH Histo kit: The kit (DF500-20XE) contains the following reagents.

i) **EZ AR™ 2 (HX032-YAX) 5ml**: One vial of EZ AR™ 2.Use upto 100ul/slide.

ii) **eFISH Liquid Pepsin (HK632-07X) 7ml:** One vial of liquid pepsin . Liquid pepsin should be kept at 37ºC for 30 minutes before use. Use upto 200ul/slide. Keep pepsin at -20°C after receiving the kit.

iii) **eFISH wash buffer 1 (10X) (HK604-20X) 200ml:** One bottle of wash buffer 1, 10X concentrated. It should be diluted to 1X before use. For Xmatrx infinity/ELITE and Xmatrx Nano 2L and 1 L can be prepared and filled in respective carboys.

iv) **eFISH Reagent A (HK972-YCX) 12ml:** One vial of eFISH Reagent A. Use upto 100ul/slide.

Note: We do not recommend the substitution of reagents 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 pipette 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.

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.

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 kit is to be stored at 2-8°C**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 REQUIRED BUT NOT SUPPLIED

S.No .	Product Name	Pack Size	Cat #
1	Barrier Slides 18x18mm	72 slides/box	XT128-SL
2	Barrier Slides 18x18mm	1440 slides/case	XT128-CL
3	Coverslips 18x18mm	175 coverslip/box	XT121-YBX
4	Coverslips 18x18mm	1750 coverslip/box	XT121-XBX
5	Barrier Slide 25x25mm	72 slides/box	XT108-SL
6	Barrier Slides 25x25mm	1440 slides/case	XT108-CL
7	Coverslips 25x25mm	90 coverslip/box	XT122-90X
8	Coverslips 25x25mm	900 coverslip/box	XT122-YQX
9.	X-DeWax	1000 ml	HX015-XAK
10.	DAPI	1 ml	HK606-10K
11.	PBS	500ml	HK091-9K
12.	EZ-AR2	1000ml	HK522-XAK
13.	Temperature controlled slide heating plate		
14.	Hybridization chamber		
15.	Caliberated Thermometer		
16.	Coplin Jars		
17.	Rubber Cement for sealing/PAP pen		
18.	Variable micro-pipettes with volumes ranging from 1 µl to 1 ml, calibrated		
19.	Tips (1-10ul, 10-200ul, 100-1000ul)		
20.	pH meter, calibrated		
21.	Timer		
22.	Forceps		
23.	Gloves		
24.	4°C Freezer		
25.	Freezer -20°C (±5°C)		
26.	Wash bottle		
27.	Tissue Paper		

Note:
BioGenex EZ-Retriever can be used for retrieval and BioGenex Xmatrx® Mini workstation can be used for complete run.

eFISH Probe/s*
Fluorescence Microscope with appropriate filter set.
Deionized water, reagent grade
Absolute ethanol

*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: It is important to avoid contamination with nucleases during all the operations.

A. PREPARATION OF REAGENTS
(See handling precautions, Section IV.)

I. eFISH wash buffer 1 (10X) should be diluted to 1X with deionized water before use. For Xmatrx infinity and Xmatrx Nano 2L and 1 L can be prepared and filled in respective carboys.

B. PROBES:
eFISH probes are recommended to use with this kit. Probes are supplied as RTU and DO NOT require any further dilution.

C. TISSUE FIXATION
The eFISH Histo kit 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 Pepsin and may result in weak or no staining. Tissue processing conditions should be standardized for consistent, reliable results. Use of a positive control probe is recommended to assess tissue processing. 4-5 µ freshly cut and overnight baked sections are recommended for optimum results. Less than 2 year old fixed tissues are recommended.

D. STAINING PROCEDURE

I. Summary of the FISH-Histo staining Protocol for automation

Sr #	Step	Reagent	Incubation Time (min) and Temperature (ºC)	No. of Incubations
	Baking	**N/A**	**20 min, 70 ºC**	**1**
1	**Dewaxing**	**X-DeWax™**	**4 min, RT**	**3**
		DI Water	**30 sec, RT**	**2**
		Alcohol	**2min, RT**	**2**
		Heat slide	**30 sec, 45 ºC**	**1**
2	**Pre-treatment**	**EZ-AR™ 2**	**N/A**	**N/A**
		Apply oil-seal&coverslip	**N/A**	**N/A**
		Heat Slide	**25min, 95-100 ºC**	**1**
		Remove coverslip/seal	**N/A**	**N/A**
		eFISH Wash Buffer 1	**1 sec, RT**	**2**
		DI water	**3 min, RT**	**2**
		Blow slide	**N/A**	**2**
		Heat slide	**1 min, 45 ºC**	**1**
3	**Pepsin Digestion**	**eFISH Liquid Pepsin**	**10-20 min, 37°C**	**1**
		eFISH Wash Buffer 1	**30 sec, RT**	**2**
		DI Water	**3 min, RT**	**2**
		Alcohol	**2 min, RT**	**2**
		Heat slide	**1 min, 45 ºC**	**1**
4	**Probe**	**eFISH probe**	**N/A**	**N/A**
		Apply oil-seal&coverslip	**N/A**	**N/A**
		Denaturation and Hybridization (varies)	**5 min, 85 ºC 16-18 hours, 37 ºC**	**1**
		Remove coverslip/seal	**N/A**	**N/A**
		eFISH Wash Buffer 1	**5 sec, RT**	**2**
		eFISH Wash Buffer 1	**1 min, RT**	**2**
5	**Stringency wash**	**eFISH Reagent A**	**2 min, 65 ºC**	**1**
		eFISH Wash Buffer 1	**10 sec, RT**	**1**
		DI water	**30 sec, RT**	**2**
		Alcohol	**45 sec, RT**	**2**
		Heat slide	**1 min, 45 ºC**	**2**
6.	**DAPI**	**DAPI**	**N/A**	**N/A**
		Coverslip/seal	**10 min, RT**	**1**

Note: To observe the slides under fluorescent microscope, seal the coverslip onto the slide using Rubber Cement /or Transparent Nail Paint/or Xmount/or DPX to avoid slipping of coverslip.

*The pepsin digestion time need to be adjusted for each tissue type. The proper digestion depends on the tissue type, thickness of tissue section and nature and duration of tissue fixation. Most FFPE tissue type yield optimal digestion between 10-16 min however conducting a pilot test with pretreatment and pepsin digestion followed by DAPI staining to find out the best optimal condition is recommended.

II. Manual FISH-Histo staining Protocol

1. For FFPE tissues: Bake the tissue at 70°C for 20mins.
2. Deparaffinization and Re-hydration:

Xylene I -	5-10 min
Xylene II -	5-10min
Xylene III -	5-10min
Alcohol 100% -	5 min
Alcohol 95% -	5 min
Alcohol 70% -	5 min

Rinse/Wash the slides to under running tap water for 5-10 minutes.

For FROZEN tissues:

1. Pre-cool the fixative (acetone, methanol or ethanol) at -20°C for 30 min.
2. Fix the tissue section with the pre-cooled fixative for 5 -10 min, at room temperature
3. Rinse 3-4 times in PBS.

3. **NUCLEIC ACID RETRIEVAL:** Decant the residual water from the slide, make the hydrophobic barrier around the tissue and add 70-100µl (depending on the tissue size) of EZ-AR™ 2.
4. Insert a cover slip carefully on the tissue and incubate for 25mins at 98°C.
   Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
   The customer can follow the following options if they do not have temperature controlled heating plate
   Option 1: Retrieval using water bath with BioGenex EZ-AR2 (HK522-XAK)
   a. Take 50ml ready-to-use EZ-AR2 solution in a Coplin jar into a programmable, room temperature, circulating water bath. and set the temperature at 98°C
   b. Monitor the temperature inside the jar using a calibrated thermometer to ensure that the temperature reaches a minimum of 95°C without reaching the boiling point of 100°C.
   c. Transfer the de-paraffinised slides into the jar and allowed to stand for 20-25mins.
   d. After completion, allow the slides to cool in the solution for 15 minutes at room temperature.
   e. Follow wash steps.
   Option 2: Using BioGenex EZ-Retriever® with BioGenex EZ-AR2 (HK522-XAK)
   a. Transfer the de-paraffinised slides into probe tank filled with EZ-AR2 solution.
   b. Set the protocol 98°C for 10mins for twice. Start the procedure
   c. After completion, allow the slides to cool in the solution for 15 minutes at room temperature.
   d. Follow wash steps.
   Note-For FROZEN tissue step 3-4 is not required.
5. Remove the cover slip with forcep very carefully.
6. Wash 3 times with eFISH wash buffer for 30 seconds.
   ENZYME DIGESTION: Decant the residual liquid properly and then add 100-200µl Pepsin; incubate for 10-20mins at 37°C.
   Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
   If Pepsin digestion is not proper repeat step 6 one more time.
7. Wash 3 times with eFISH wash buffer for 30 seconds.
8. Decant the residual water properly and then add alcohol and incubate for 1min.
9. Decant the alcohol properly and dry.
10. **PROBE HYBRIDIZATION:** Add 10-20µl BioGenex FISH Probe; insert cover slip and hybridize for 5mins at 85°C. Please maintain dark environment.
    Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
11. Seal with rubber cement.
12. Incubate for 18 hrs at 37°C in **DARK** humidified condition.
    Note: Probe incubation depends on Probe specification.
    Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
13. Remove the cover slip with forcep very carefully.
14. Wash 4 times with eFISH wash buffer for 30 seconds. Decant the residual buffer properly.
15. Add 100-200 ul eFISH Reagent A and incubate for 2mins at 65°C, 1min at 25°C.
    Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
    Wash 2 times with eFISH wash buffer for 30sec, 2 times with deionised water and 2 times with alcohol for 30sec respectively.
16. Air dry the slide for complete removal of residual alcohol.
17. **MOUNT:** Add 10-15µl DAPI very slowly to the middle of the tissue and slowly mount with cover slip.
18. Keep the slide in RT for 10min in dark condition and observe under Fluorescence Microscope.
19. Store the slide at -20°C for future use.

VII. EXPECTED RESULTS

Proper use of this detection kit will result in an intense fluorescence signal stain at the specific site of the hybridized probe. Nucleus can be visualized with DAPI staining. Appropriate filter sets should be used according to the fluorescence probes used. Slides should be read by a qualified pathologist/cytogenetist. The interpretation of any test results is solely the responsibility of the user.

VIII. QUALITY CONTROL

Each Fluorescence *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. REFERENCES

1. Gall, J. G. and Pardue, M. L. (1969). Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proc. Natl. Acad. Sci. USA* 63,378 -383.
2. Rudkin, G. T. and Stollar, B. D. (1977). High resolution detection of DNA RNA hybrids *in situ* by indirect immunofluorescence. *Nature* 265,472-473.
3. Hougaard, D. M., Hansen, H. and Larsson, L. I. (1997). Non-radioactive *in situ* hybridization for mRNA with emphasis on the use of oligodeoxynucleotide probes. *Histochem. Cell Biol.* 108,335 -*344.*
4. Bauman, J. G., Wiegant, J., Borst, P. and van Duijn, P. *(*1980*).* A new method for fluorescence microscopical localization of specific DNA sequences by *in situ* hybridization of fluorochromelabelled RNA. *Exp. Cell Res.* 128,485 -*490.*
5. O'Connor, C. (2008) Fluorescence *in situ* hybridization (FISH). *Nature Education* 1(1):171

2ºC 8ºC	Temperature Limitation	IVD	In Vitro Diagnostic Medical Device
	Use By Date	LOT	Batch Code
NON STERILE	Non-Sterile		Consult Instructions for Use
EC REP	Representative in the European Community		Manufacturer