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eFISH Cyto
Cat. No. DF510-20XE

Doc. No. 932-DF510-20XE Rev. No.: C
Release Date: 18-Oct-2021

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

For In Vitro Diagnostic Use

I. INTENDED USE

eFISH Cyto kit is intended for use in an Fluorescence *in Situ* Hybridization (FISH) procedure and 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 mRNA sequences. The eFISH Cytology kit is designed for use with slides prepared from cytological/hematological specimens/smears/cells. Standard laboratory procedure should be used while processing cytology/hematology specimens. A de-calcification step may be required for the preparation of bone marrow sample. 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 technique of cytogenetic used for the detection of chromosomal aberrations viz., viz., deletions, amplification, duplication and translocation in tissue sections or within individual cells in native context. In this technique florescent probes bind to the target sequence of DNA in chromosome. High specificity and sensitivity coupled rapid and accurate result has proven role of FISH in both research and diagnosis of solid tumor and hematological malignancies. 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/cytology specimens/smears are pretreated to expose target DNA or mRNA sequences. An appropriately labeled probe is hybridized to the exposed target in the cells, stringency washing steps remove non-specifically bound probe. 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 cytology kit is a novel system for processing of cytological/hematological specimens/smears/cells. This kit contains reagents for pretreatment and post-hybridization stringency washings of the tissue. This kit is to be used for BioGenex eFISHciency FISH processing platforms Xmatrx Infinity/Elite, Xmatrx Nano and Xmatrx Mini along with BioGenex eFISH probes. Also this kit can be used for manual procedure. eFISH probes can be ordered separately, complete details of eFISH probes is available on our company website.

BioGenex eFISH Cytology kit: The kit (DF510-20XE) contains the following reagents.

i) **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.

ii) **eFISH Formalin fixative (HK603-20X) 20ml:** One vial of eFISH formalin fixative solution. Use upto 200ul/slide.

iii) **eFISH wash buffer 1 (10X) (HK604-20X) 200ml:** One bottle of wash buffer1 10X. It should be diluted to 1X before use. For Xmatrx infinity and Xmatrx Nano 2L and 1 L can be prepared and filled in respective carboys.

iv) **eFISH wash buffer 2 (10X) (HK605-20X) 200ml:** One vial of wash buffer 2, 10X. It should be diluted to 1X before use. For Xmatrx infinity and Xmatrx Nano 2L and 1 L can be prepared and filled in respective carboys.

Note: It is recommended that the reagents may not be substituted 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 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.

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 20-26°C except liquid pepsin (-20°C). If reagents are stored under any conditions other than those specified in the package insert, performance must be verified by the user. Upon arrival please store pepsin at -20°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 NEEDED BUT NOT SUPPLIED

Sr #	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	DAPI	1 ml	HK606-10K
10	PBS	500ml	HK091-9K
11	Temperature controlled slide heating plate		
12	Hybridization chamber		
13	Caliberated Thermometer		
14	Coplin Jars		
15	Rubber Cement for sealing/PAP pen		
16	Variable micro-pipettes with volumes ranging from 1 µl to 1 ml, calibrated		
17	Tips (1-10ul, 10-200ul, 100-1000ul)		
18	pH meter, calibrated		
19	Timer		
20	Forceps		
21	Gloves		
22.	4°C Freezer		
23	Freezer -20°C (±5°C)		
24	Wash bottle		
25	Tissue Paper		

Note:
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 all the operation should be

A. PREPARATION OF REAGENTS

(See handling precautions, Section IV.)

I. eFISH wash buffer 1 and 2 should be diluted to 1X with deionzed 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. SLIDE PREPARATION

The eFISH Cytology kit is designed for use with slides prepared from cytological**/hematological specimens/smears/cells.** Standard laboratory procedure should be used while processing cytology/hematology specimens. A de-calcification step may be required for the preparation of bone marrow sample. BioGenex barrier slides compatible for respective automation platform should be used for preparation of these specimens.

D. STAINING PROCEDURE

I. Summary of the FISH-Cyto staining Protocol on Automation

Sr #	Step	Reagent	Incubation Time (min)* and Temperature (ºC)	No. of Incubations
1	**Formalin Fixation**	eFISH Formalin fixative	15 min,RT	NA
		eFISH Wash buffer2	30 sec, RT	1
		eFISH Wash buffer2	5 min, RT	1
2	**Ethanol Wash**	Alcohol Wash	30 sec, RT	1
		Alcohol Wash	2 min, RT	1
3	**Aging operation/Pre-treatment**	eFISH wash buffer1	2 min,60 ºC	1
		eFISH Wash buffer2	30 sec, RT	1
		eFISH Wash buffer2	1 min, RT	1
4	**Pepsin Digestion**	eFISH Liquid Pepsin	15 min, 37°C	1
		eFISH Wash buffer2	30 sec, RT	1
		eFISH Wash buffer2	5 min, RT	1
		Alcohol Wash	2 min, RT	2
5	**Probe**	eFISH probe	NA	NA
		Apply oil/seal/coverslip	N/A	N/A
		Denaturation and Hybridization	85 ºC, 5 min 37 ºC, 16-18hrs	1
6	**Stringency wash**	Remove coverslip/seal	N/A	N/A
		eFISH Wash buffer2	30 sec, RT	2
		eFISH wash buffer 1	50 ºC, 2min	1
		eFISH Buffer 2	1 sec, RT	2
		DI water	30 sec, RT	2
		Alcohol	45 sec, RT	2
		Heat slide	45 ºC, 1 min	1
7	**DAPI**	DAPI	N/A	1
		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 specimen type. The proper digestion depends on the specimen type and slide preparation procedure. Most specimen 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-Cyto staining Protocol

1. Prepare the slides from cytological/hematological specimens /smears / cells using standard laboratory procedures.
2. FIXATION: The cells may be fixed using one of two methods:
   A. Incubating the cells in 100% methanol (chilled at -20°C) at room temperature for 5 min.
   B. Using 4% paraformaldehyde in PBS pH 7.4/Formalin for 10-15 min at room temperature.
3. The cells should be washed three times with ice-cold PBS (A)/two times with eFISH Buffer 2(B) for 2-3min.
4. Wash two times with alcohol for 2-3 mins.
5. Incubate the slides for 2-3mins with eFISH wash buffer 1 at 60°C.
Note:
Option 1:
a. Take 50ml eFISH wash buffer 1 in a Coplin jar into a programmable, room temperature, circulating water bath.and set the temperature at 60°C.
b. Monitor the temperature inside the jar using a calibrated thermometer to ensure that the temperature reaches a minimum of 60°C.
Option 2:
Use hybridization Chamber
6. Incubate the slides for 2min with eFISH wash buffer 2 at room temperature and repeat this step again.
7. ENZYME DIGESTION:
a. Decant the residual liquid properly and then add 100-200µl Pepsin; incubate for 10-20mins at 37°C.
Note: Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber also for this particular step.

Note: If Pepsin digestion is not proper repeat step 6 one more time.
8. Wash the slides two times with eFISH wash buffer 2 at room temperature for 3mins.
9. Decant the residual buffer properly and wash two times with alcohol for 2 mins.
10. Decant the alcohol and dry the slides.
11. PROBE HYBRIDIZATION:
Add 10µl BioGenex FISH Probe; apply 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.
12. Seal with rubber cement.
13. Incubate for 16-18 hrs at 37°C in **DARK** humidified condition.
Note: Probe incubation time depends on Probe specifications.
Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
14. Remove the cover slip with forcep very carefully.
15. Wash 3 times with eFISH wash buffer 2 for 1-2mins at room temperature. Decant the residual buffer properly.
16. Incubate with eFISH wash buffer 1 for 2mins at 50°C.
Temperature controlled slide heating plate needs to be used for this step. One can use hybridization chamber for this particular step.
17. Wash 2 times with eFISH wash buffer 2 for 30sec, 2 times with deionized water, and 2 times with alcohol for 30sec respectively at room temperature.
18. Air dry the slide for complete removal of residual alcohol.
19. MOUNT: Add 10-15µl DAPI very slowly to the middle of the tissue and slowly mount with cover slip.
20. Keep the slide in RT for 10min in dark condition and observe under Fluorescence Microscope.
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.

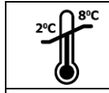
- **Positive control probe:** The positive probe is known to be complementary to nucleic acid sequences in a test tissue slide that is processed in a manner identical to the slides that are being tested.
- **Negative control probe:** A negative probe is known not to be complementary to the target nucleic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

	Temperature Limitation		In Vitro Diagnostic Medical Device
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