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eFISH Cyto Kit For NanoVIP® Cat. No. DF530-20X

Doc. No. 932-DF530-20X

Rev. No.: B

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**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 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 platform NanoVIP® along with BioGenex eFISH probes. eFISH probes can be ordered separately, complete details of eFISH probes is available on our company website.

BioGenex eFISH Cytology kit: The kit (DF530-20X) contains the following reagents.

i) **eFISH Liquid Pepsin (HX632-06X):** 1 x 6ml of liquid pepsin . Liquid pepsin should be kept at 37°C for 30 minutes before use.

ii) **eFISH Formalin fixative (HX603-08X):** 8 ml eFISH formalin fixative solution.

iii) **eFISH wash buffer 1 (10X) (HX604-20X):** 200ml of wash buffer1 10X. It should be diluted to 1X before use. For For NanoVIP® 1 L can be prepared and filled in respective carboys.

iv) **eFISH wash buffer 2 (10X) (HX605-20X):** 200ml of wash buffer 2, 10X. It should be diluted to 1X before use. For For NanoVIP® 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	70 slides/box	XT128-SL
2	Barrier Slides 18x18mm	1400 slides/case	XT128-CL
3	Coverslips 18x18mm	175 coverslip/box	XT121-YBX
4	Coverslips 18x18mm	1750 coverslip/box	XT121-XBX
5	Barrier Slide 25x25mm	70 slides/box	XT108-SL
6	Barrier Slides 25x25mm	1400 slides/case	XT108-CL
7	Coverslips 25x25mm	90 coverslip/box	XT122-90X
8	Coverslips 25x25mm	900 coverslip/box	XT122-YQX
9.	EZ-DeWax™		HK584-5KE

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 deionized water before use. For For NanoVIP® 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

Summary of the FISH staining Protocol

Sr #	Step	Reagent	Incubation Time (min)* and Temperature (°C)	No. of Incubations
1	Ethanol Wash	Alcohol Wash	30 sec, RT	1
		Alcohol Wash	2 min, RT	1
2.	Aging operation	eFISH wash buffer1	2 min,60 °C	1
		eFISH Wash buffer2	30 sec, RT	1
3	Pepsin Digestion	eFISH Wash buffer2	1 min, RT	1
		eFISH Liquid Pepsin	15 min, 37°C	1
		eFISH Wash buffer2	30 sec, RT	1
		eFISH Wash buffer2	5 min, RT	1
4	Formalin Fixation	eFISH Formalin fixative	15 min,RT	
		eFISH Wash buffer2	30 sec, RT	1
		eFISH Wash buffer2	5 min, RT	1
4	Probe	Alcohol Wash	2 min, RT	3
		eFISH probe	NA	NA
		Apply oil/seal/coverslip	N/A	N/A
5	Stringency wash	Denaturation and Hybridization	85 °C, 8 min 37 °C, 17hrs	1
		Heat slide	40 °C, 2 min	1
		Remove	N/A	N/A

		coverslip/seal	
	eFISH Wash buffer2	30 sec, RT	2
	Heat slide	45 °C, 30 sec	1
	eFISH wash buffer 1	50 °C, 1 min 30 sec	1
	eFISH Buffer 2	1 sec,RT	2
	Heat slide	45 °C, 1 min	1
6.	DAPI	DAPI	N/A
	Coverslip/seal	10 min, RT	1

*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.

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. Hougard, 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