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eFISH Assay Kit
Cat. No. DF720-20X

Doc. No: 932-DF720-20X Rev. No. B
Release Date: 18-Oct-2022

For Research Use Only

I. INTENDED USE

eFISH Assay Kit is intended for use in a single molecule fluorescence *in situ* hybridization (smFISH) procedure and is optimized and designed for the multiplexed detection of mRNA. The technique uses fluorescent labeled nucleic acid probes following hybridization to target 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.

II. PRINCIPLES OF THE PROCEDURE

smFISH is a robust technique that uses fluorescent probes to detect specific mRNA molecules in their native context. In this technique, multiple oligo-conjugated probes bind to the target mRNA. Fluorescent labeled probes complementary to the oligo-conjugated probes are then hybridized to detect the target. This kit is designed in such a way to amplify the signal from a single target by using multiple probes. High specificity and sensitivity coupled with rapid and accurate results of smFISH have proven to be useful in both research and diagnosis of solid tumor and hematological malignancies. As a technique of cancer diagnosis, smFISH can be used to accurately determine the abundance and localization of mRNAs that are proved to have a role in disease progression. smFISH can also be used to detect and localize specific mRNA targets in cells, circulating tumor cells, and tissue samples.

The Omicsveu eFISH Assay Kit is designed for smFISH procedure. Initially, multiple oligo-conjugated probes bind to the target mRNA in the tissue sections. Then, an appropriately labeled probe complementary to the DNA sequence of the oligo-barcode is hybridized. Subsequent stringent washing steps remove any complementary probe that is non-specifically bound to the tissue section. Finally, slides are mounted using DAPI and can be visualized under fluorescence microscope using appropriate filter set. After each cycle and image acquisition, previously bound oligo-conjugated probes are stripped from the tissue. The same tissue can be used for 8-10 cycles (recommended/based on the tissue quality) of smFISH, allowing the detection of multiple targets in a single tissue section.

III. REAGENTS AND MATERIALS SUPPLIED

The Omicsveu eFISH Assay Kit is a novel system for detecting target mRNAs in FFPE tissues. This kit contains reagents for pretreatment, post-hybridization stringency washing, and probe stripping of the tissue. This kit can be used for performing smFISH assay on BioGenex NanoVIP® platform along with Omicsveu oligo-conjugated probes. Additionally, this kit can also be used for manual smFISH procedure. Oligo-barcoded probes and their complementary probes can be ordered separately. Complete details of the same are available on our company website.

Omicsveu eFISH Assay Kit: The kit (DF720-20X) contains the following reagents:

i) **EZ AR™ 2 (HX032-04X), 4 mL**: One vial of EZ AR™ 2. Use up to 100 µL/slide.

ii) **eFISH Liquid Pepsin (HX632-06X), 6 mL:** One vial of liquid pepsin . Liquid pepsin should be kept at 37 ºC for 30 min before use. Use up to 200 µL/slide. Store pepsin at -20 °C upon receiving the kit.

iii) **eFISH wash buffer 1 (10X) (HX604-20X), 200 mL:** One bottle of concentrated wash buffer 1 (10X). It should be diluted to 1X using deionized water before use.

iv) **eFISH Reagent A (HX972-08X), 8 mL:** Two vials of eFISH Reagent A. Use up to 100 µL/slide.

v) **DAPI (HK606-10K), 1 mL:** One vial of DAPI. Use up to 10 µL/slide.

vi) **Ribonucleoside vanadyl complexes (HK996-20), 0.5 mL:** One vial of ribonucleoside vanadyl complexes. Store at -20 °C as soon as the kit is received.

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 should be disposed of with proper caution.

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 (However, pepsin and ribonucleoside vanadyl complexes solution should be stored in -20 °C upon receiving).

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 18x18 mm	72 slides/box	XT128-SL
2	Barrier Slides 18x18 mm	1440 slides/case	XT128-CL
3	Coverslips 18x18 mm	175 coverslip/box	XT121-YBX
4	Coverslips 18x18 mm	1750 coverslip/box	XT121-XBX
5	Barrier Slide 25x25 mm	72 slides/box	XT108-SL
6	Barrier Slides 25x25 mm	1440 slides/case	XT108-CL
7	Coverslips 25x25 mm	90 coverslip/box	XT122-90X
8	Coverslips 25x25 mm	900 coverslip/box	XT122-YQX
9.	EZ-DeWax™	1000 mL	HK585
11.	Phosphate buffer saline (PBS) buffer	500 mL	HK091-9K
12.	EZ-AR2	1000 mL	HK522-XAK
13.	Temperature controlled slide heating plate		
14.	Hybridization chamber		
15.	Calibrated thermometer		
16.	Oligo-barcoded probes*		
17.	Nuclease free water		

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

*These products are available from Omicsveu. Please refer to Omicveu catalog for details or contact Omicsveu 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)
eFISH wash buffer 1 (10X) should be diluted to 1X with deionized water before use.

B. OLIGO-BARCODED ANTIBODIES

Oligo-barcoded probes and complementary probes are recommended to use with this kit. They are supplied as ready-to-use and DO NOT require any further dilution.

C. TISSUE FIXATION

The eFISH Assay Kit is designed for use with FFPE tissue sections. For best results, specimens should be fixed in 10% neutral buffered formalin for 5–20 h. Tissue processing conditions should be standardized for consistent and reliable results. Use of a positive control is recommended to assess tissue processing. Freshly cut (4–5 micron) and overnight baked sections are recommended for optimum results. Less than 2 year old fixed tissues are recommended.

D. STAINING PROCEDURE

1. Summary of eFISH staining protocol for automation

S.No.	Step	Reagent	Incubation Time (min) and Temperature (ºC)	No. of Incubations
1	Baking	N/A	30 min, 70 ºC	1
		N/A	1 min, 27 ºC	1
2	Dewaxing	X-DeWax™	5 min, RT	5
		Alcohol	30 s, RT	2
		DI Water	30 s, RT	2
		Blow slide	N/A	1
		Heat slide	1 min, 40 ºC	1
3	Pre-treatment	EZ-AR™ 2	N/A	N/A
		Apply oil-seal and coverslip	N/A	N/A
		Heat Slide	5 min, 85 ºC	1
		Heat Slide	20 min, 95 ºC	1
		Heat Slide	3 min, 25 ºC	1
		Remove coverslip	N/A	N/A
		DI water	30 s, RT	4
		Heat slide	1 min, 40 ºC	1
		Blow slide	N/A	2
4	Pepsin digestion*	eFISH Liquid Pepsin	10–20 min, 37 °C	1
		Blow slide	N/A	1
		DI Water	30 s, RT	2
		Alcohol	30 s, RT	1
		DI Water	30 s, RT	2
		Heat slide	2 min, 45 ºC	1
5	Probe	Oligo-conjugated mRNA probe	N/A	N/A
		Apply oil-seal and coverslip	N/A	N/A
		Denaturation and Hybridization (varies)	10 min, 55 ºC; 6 h, 45 ºC	1
		Remove coverslip	N/A	N/A
	Stringency wash	eFISH Reagent A	1 min, 55 ºC	1
		eFISH Wash Buffer 1	30 s, RT	3
		Blow slide	N/A	2
	Complementary probe	Fluorescent probe	N/A	N/A
		Apply oil-seal and coverslip	N/A	N/A
		Hybridization	4 h, 37 ºC	1
		Remove coverslip	N/A	N/A
		eFISH Wash Buffer 1	30 s, RT	4
6	Stringency wash	eFISH Reagent A	1 min, 55 ºC	1
		Blow slide	N/A	1
		eFISH Wash Buffer 1	30 s, RT	3
		Blow slide	N/A	2
		Heat slide	2 min, 45 ºC	1
		Blow slide	N/A	1
7	DAPI	DAPI	N/A	N/A
		Apply oil seal and coverslip	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.

2. Protocol for manual eFISH staining

Please follow the link given below for manual eFISH staining protocol:
https://omicsveu.com/wp-content/uploads/Brochures/914-0083.0.pdf

3. Protocol for stripping of probes

3.1. Stripping protocol

After each cycle of smFISH and image acquisition, bound probes should be stripped before next cycle. Following is the protocol for probe stripping from the tissue (can be performed manually or using BioGenex platforms such as Xmatrx Infinity/ELITE, Nano VIP, and Xmatrx Nano):

S.No.	Step	Reagent	Incubation Time (min) and Temperature (ºC)	No. of Incubations
1.	Incubation	Stripping buffer*, 100 µL	5 min, 90 °C	1
2.	Stringency Wash	eFISH Reagent A, 100 µL	2-3 min, 65 °C	1
		eFISH Wash Buffer 1	30 s, RT	2
3	Incubation	Stripping buffer*, 100 µL	5 min, 80 °C	1
4	Stringency Wash	eFISH Reagent A, 100 µL	2-3 min, 65 °C	1
		eFISH Wash Buffer 1	30 s, RT	2
5	Repeat steps 3 and 4			

* Stripping buffer has to be prepared freshly.

2.2. Stripping buffer preparation:

The following table provides the reagents required to make 5 mL of stripping buffer.

Reagent	Volume (mL)
Nuclease free water*	4.45
10X eFISH wash buffer 1	0.5
Ribonucleoside vanadyl complexes	0.05

* Nuclease free water is not supplied with the kit

VII. EXPECTED RESULTS

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

VIII. QUALITY CONTROL

Each eFISH assay should include control slides to confirm that the detection kit is working properly and that the protocol followed has worked.

IX. Preparation of the 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: A positive control is known to bind to target mRNA in a test tissue slide that is processed in a manner identical to the slides that are being tested.

Negative control: A negative control is known not to bind to the target that is being detected.

X. 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 the tissue. The results from smFISH must be correlated with other laboratory findings.

XI. REFERENCES

1. 10.1073/pnas.63.2.378
2. 10.1038/265472a0
3. 10.1007/s004180050174
4. 10.1016/0014-4827(80)90087-7
5. nature.com/scitable/topicpage/fluorescence-in-situ-hybridization-fish-327/
6. 10.1007/978-3-642-16712-6_518

8°C 2°C	Temperature Limitation	RUO	For Research Use Only
	Use By Date	LOT	Batch Code
NON STERILE	Non-Sterile		Consult Instructions for Use
REF	Catalogue Number		BioGenex

Distributed by: OmicsVeu

Omicsveu is a distributor company offering the true spatial multi-omics platform & reagents.

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