BioGenex
48810 Kato Road, Suite 100E & 200E,Fremont, CA 94538
Tel : +1 (800) 421-4149, Fax: +1 (510) 824-1490,
support@biogenex.com

DATA SHEET

eFISHSPEC 13/CEN 18/SPEC 21 TripleColor probe

Catalog No.
FP096-10X-100µl- 10 test
FP096-20X -200µl- 20 test

Doc No: 932-FP096 Rev: B
Date of Release: 10-Aug-2020

Material Provided: One vial of eFISH probe in hybridization buffer (RTU).

Recommended detection system (Not supplied):
Either of the following detection systemsis recommended depending on the automation/manual platform used:

eFISH Kit	Cat #	Description
eFISH Histo	DF-500-20X	Automation
eFISH Cyto	DF-510-20X	Automation

Intended Use:
The BioGenex eFISH SPEC 13/CEN 18/SPEC 21 TripleColor probeis currently available for Research use only.eFISHSPEC 13/CEN 18/SPEC 21. TripleColor probeis designed to be used forthe detection of human chromosome 13q12specific sequences as well as chromosome 18alphasatellites and chromosome 21q22 specificsequences in formalin-fixed, paraffin-embeddedtissue or cells by fluorescence in situ hybridization(FISH).

The BioGenex eFISH SPEC 13/CEN 18/SPEC 21 TripleColor probe in hybridization buffer. The probecontains green-labeled polynucleotides (Green:excitation at 503 nm and emission at 528 nm,similar to FITC), which target chromosome 13specific sequences, blue-labeled polynucleotides(Blue: excitation at 418 nm and emission at467 nm, similar to DEAC) which target alpha-satellitesequences of the centromere of chromosome18, and orange-labeled poly-nucleotides(Orange: excitation at 547 nm and emission at572 nm, similar to rhodamine), which targetchromosome 21 specific sequences.

Summary and Explanation
Fluorescence *in situ* hybridization (FISH) is a robust technique of cytogenetic 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 in chromosome. High specificity and sensitivity coupled rapid and an accurate result has proven 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 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 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.

Principles of the Procedure
In Situ hybridization (ISH) allows the detection and localization of definitive nucleic acid sequences directly within a cell or tissue. High specificity is ensured through the action of annealing of fluorescence probe nucleic acid sequence to complementary target nucleic acid sequence. ISH techniques can be used to identify genetic aberrations like deletions, amplification, and translocationin tissue sections or within individual cells.

Storage and Handling
The BioGenex eFISH SPEC 13/CEN 18/SPEC 21 TripleColor probemust be stored at 2-8°C protected from light and is stable through the expiry date printed on the label.

Specimen Collection and Slide Preparation
Tissues fixed in 10% (v/v) formalin are suitable for use prior to paraffin embedding and sectioning.

FISH Staining procedure
- **(a)** The BioGenex eFISH probes are supplied in hybridization buffer and used without further dilution.
- (b) Protocol:

Please refer to the eFISH probe specific instruction/protocol for automated or semi-automated FISH processing platform (Xmatrx®-Infinity, Xmatrx®-Nano and Xmatrx®-mini.

Further processing, such as washing and counter-staining, can becompleted according to the user's needs. For a particularly user-friendlyperformance, we recommend the use of a BioGenexeFISH kit.

Disclaimer: The above information is provided for reference only. Each end-user is

BioGenex
48810 Kato Road, Suite 100E & 200E,Fremont, CA 94538
Tel : +1 (800) 421-4149, Fax: +1 (510) 824-1490,
support@biogenex.com

responsible for developingand validating optimal testing conditions for use with this product.

Troubleshooting
Contact BioGenex Technical Service Department at **1-800-421-4149 or your local distributor** to report unusual staining.

Expected Results
With the use of BioGenex eFISH SPEC 13/CEN 18/SPEC 21 TripleColor probe , thehybridization signals of labeledchromosome 13 specific sequences appear green; the hybridizationsignals of labeled alpha-satellite-sequences of the centromere ofchromosome18 appear blue, and the hybridization signals of labeled chromosome21specific sequences appear orange. In interphases of normal cellsor cells withoutaberrations of chromosomes 13, 18, and 21, two chromosome13, two chromosome 18, and two chromosome 21 signalsappear. In cells with an aneuploidy of one of thechromosomes mentionedabove, a different signal pattern is visible in interphases.

In order to judge the specificity of the signals, every hybridization shouldbe accompaniedby controls. We recommend using at least one controlsample in which the chromosome 13, 18, and 21 copy number is known.

Care should be taken not to evaluate overlapping cells, in order to avoidfalse results, e.g. an amplification of genes. Due to decondensedchromatin, single FISH signals can appear as small signal clusters. Thus,two or three signals of the same size, separated by a distance equal to orless than the diameter of one signal, should be counted as one signal.

Limitations of the Procedure
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.

Bibliography

1. Gall, J. G. and Pardue, M. L. (1969). *Proc. Natl. Acad. Sci. USA*63, 378 -383.
2. Rudkin, G. T. and Stollar, B. D. (1977). *Nature* 265,472-473.
3. Hougaard, D. M., Hansen, H. and Larsson, L. I. *(*1997*). Histochem. Cell Biol.* 108,335 -*344.*
4. Bauman, J. G., Wiegant, J., Borst, P. and van Duijn, P. *(*1980*).. Exp. Cell Res.* 128,485 -*490.*
5. O'Connor, C. (2008). *Nature Education* 1(1):171.