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

eFISH EGFR/CEN7 Dual Color Probe

Catalog No.
FP040-10X-100µl- 10 test
FP040-20X-200µl- 20 test

Doc No: 932-FP040 Rev: C
Date of Release: 05-Aug-2020

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

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

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

Intended Use:
The BioGenex eFISH EGFR/CEN7 dual color probe is currently available for Research use only. eFISH EGFR/CEN7 dual color probe is designed to be used for the detection of human EGFR2 geneas well aschromosome 7 alpha-satellites in formalin-fixed, paraffin-embedded tissue or cells by fluorescence in situ hybridization (FISH).

BioGenex eFISH EGFR/CEN7dual color probecomes in hybridization buffercontainsgreen-labeled polynucleotides (Green:excitation at 503 nm and emission at 528 nm,similar to FITC), which target the EGFR gene, andorange-labeled polynucleotides (Orange:excitation at 547 nm and emission at 572 nm,similar to rhodamine), which target alphasatellite-sequences of the centromere ofchromosome 7.

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 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.

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, translocation in tissue sections or within individual cells.

Storage and Handling
The BioGenex eFISH EGFR/CEN7dual color probe must 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.

Protocol
Please refer to the 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 BioGenex eFISH Kit. These systems were also used for the confirmation ofappropriateness of the BioGenex eFISH EGFR/CEN7 dual color probe.

Disclaimer: The above information is provided for reference only. Each end-user is 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
The use of BioGenex eFISH EGFR/CEN7 in interphase and normal chromosome 7 produces two EGFR signals and two chromosome 7 signals. These signals appear green

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

with hybridized EGFR probe and orange when hybridized to alpha-satellite-sequences of centromere of chromosome 7, when appropriate filter sets are used. In cases of increased gene amplification in these regions, an increased number of gene signals are visible.

BioGenex eFISH EGFR/CEN7 serves as a internal control as it recognizes the alpha-satellite-sequences of the centromere of chromosome 7 to prove the integrity of the cellular DNA and the success of hybridization.

However, we recommend the use of a control sample in which the chromosome 7 and EGFR gene region copy number is known to judge the specificity of the signals with each hybridization reaction.

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*). Cell Res.* 128,485 -*490.*
5. O'Connor, C. (2008). *Nature Education* 1(1):171
6. Alexandrov IA, et al. (1988) Chromosoma 96: 443-53.
7. Cappuzzo F, et al. (2005) Natl Cancer Inst 97: 643-55.
8. Kievits T, et al. (1990) Cytogenet Cell Genet 53: 134-6.
9. Kondo I, Shimizu N (1983) Cytogenet Cell Genet 35: 9-14.
10. Libermann TA, et al. (1985) Cell SciSuppl 3: 161-72.
11. Merlino GT, et al. (1985) J Clin Invest 75: 1077-9.
12. Sassen A, et al. (2008) Breast Cancer Res 10: R2.
13. Tovey SM, et al. (2004) Breast Res 6: 246-51.
14. Waye JS, Willard HF (1986) Nucleic Acids Res 14: 6915-27.
15. Wilkinson DG: In Situ Hybridization, A Practical Approach, Oxford University Press (1992)ISBN 0 19 963327 4.