

DATA SHEET

eFISH RETdual color break apart probe

Catalog No.**FP061-10X- 100µl-10 test****FP061-20X- 200µl-20 test**

Doc No: 932-FP061 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 RET dual color break apart probe is currently available for Research use only. eFISH RET dual color break apart probe is designed to be used for the detection of translocations involving the RET gene at 10q11.21 in formalin-fixed,paraffin-embedded tissue or cells by fluorescence in situ hybridization (FISH).

BioGenex eFISH RET dual color break apart probecomes in hybridization buffer. The probe contains green-labeled polynucleotides (Green: excitation at 503 nm and emission at 528 nm, similar to FITC), which target sequences mapping in 10q11.21 distal to the RET breakpoint cluster, and orange labeled polynucleotides (Orange: excitation at 547 nm and emission at 572 nm, similar to rhodamine), which target sequences mapping in 10q11.21 proximal to the RET breakpoint cluster.

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 translocation in tissue sections or within individual cells.

Storage and Handling

The BioGenex eFISH RET dual color break apart 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

- The BioGenex eFISH probes are supplied in hybridization buffer and used without further dilution.
- 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 be completed according to the user's needs. For a particularly user-friendly performance, we recommend the use of a BioGenex eFISH kit.

These systems were also used for the confirmation of appropriateness of the BioGenex eFISH RET dual color break apart 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

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distributor to report unusual staining.

Expected Results

The BioGenex eFISH RET Dual Color Break Apart Probe is a mixture of two direct labeled probes hybridizing to the 10q11.21 band. The orange fluorochrome direct labeled probe hybridizes proximal to RET gene, the green fluorochrome direct labeled probe hybridizes distal to the RET breakpoint region at 10q11.21

The use of eFISH RET in interphase nucleus lacking a translocation involving the 10q11.21 band, two orange/green fusion signals are expected representing two normal (non-rearranged) 10q11.21 loci. A signal pattern consisting of one orange/green fusion signal, one orange signal, and a separate green signal indicates one normal 10q11.21 locus and one 10q11.21 locus affected by a translocation or inversion.

However, we recommend the use of a control sample in which the 10q11.21 status 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 avoid false results, e.g. an amplification of genes. Due to decondensed chromatin, single FISH signals can appear as small signal clusters. Thus, two or three signals of the same size, separated by a distance equal to or less 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

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