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DATA SHEET
U6 fluorescenated oligo probe

Catalog No.	Description
PR031-100E	One vial of 0.650 ml of probe in hybridization buffer

Doc No: 932- PR031-100E Rev:D
Date of Release:20-Aug-2020

Recommended detection system:
DF400-50KE-One step ISH Detection Kit- Manual Use

Intended Use:
The U6 fluorescenated oligo probe is designed to bind to human U6 small nuclear RNA in formalin-fixed, paraffin-embedded human tissues or freshly prepared frozen tissues by *in situ* hybridization. This probe does not react with normal human mRNA or nuclear DNA present in tissues. This probe can be used as positive control for *in situ* hybridization assays.

Summary and Explanation
U6 snRNA is the non-coding small nuclear RNA (snRNA) component of U6 snRNP (small nuclear ribonucleoprotein). The U6 snRNA sequence is highly conserved and the function of the U6 snRNA has remained crucial and unchanged through evolution. The U6 cellular transcript is available in abundance with intranuclear distribution in cell/tissue.

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 probe nucleic acid sequence to complementary target nucleic acid sequence. ISH techniques can be used to identify infectious agents in tissue sections, to localize gene expression within individual cells, or to detect specific nucleic acid sequences in cells.
In ISH, fixed tissue sections are treated with nucleic acid retrieval solution to expose target nucleic acid sequences. A hapten (fluorescein labeled probe) is hybridized to the exposed target nucleic acid sequences in the cells. Subsequent washing steps remove any probe that is not bound or that is non-specifically bound to the tissue section. An immunohistochemical (IHC) procedure is then used to detect the probe-target hybrid. (Downstream detection of hybridized hapten labeled probe is done by using specific anti-hapten antibody). This procedure includes incubating the slide with a mouse anti-fluorescein or digoxigenin antibody, followed by detection of this antibody with a second antibody enzyme conjugate. After addition of an appropriate substrate for the enzyme (such as DAB, diaminobenzidine solution), a brown colored reaction product is precipitated at the location of the probe-target hybrid. Microscopic examination of the slide provides visual interpretation of the staining results.

Materials Required But Not Provided
Dewax, nucleic acid retrieval solution (NAR), control slides, control reagents and other ancillary reagents are not provided.
Please refer to the package insert(s) of one step ISH detection kit for detailed protocols and instructions on use of the reagents.

Storage and Handling
Store the probe at 2-8^{o} C. The probe is allowed to reach room temperature prior to use.
This probe is suitable for use till expiry date when stored at 2-8°C. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.
Positive and negative controls should be run simultaneously for every experiment. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact BioGenex Technical Support at **1-800-421-4149 or your local distributor**.

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

Treatment of Tissues Prior to Staining
All formalin-fixed, paraffin-embedded tissue sections require pretreatment with Nucleic Acid Retrieval solution (NAR)

Staining procedure
(a) The BioGenex miRNA probes are supplied in hybridization buffer and used without further dilution.
(b) The probe solution is brought to room temperature just prior to use.
(c) All testing is done at room temperature. The testing parameters and testing protocols are listed in Table 1 below.
(d) The BioGenex one step ISH Detection Kit is used to detect hybridized probes following the instructions in the package insert for the detection system.
(e) The negative and positive control probe is run in parallel.

Recommended protocol and parameters for U6 probe
Table 1: Protocol for Manual Staining Procedure -FFPE tissue section

Reagent	Incubation time temperature	No. of DI water rinses	No. of buffer rinses
EZ-Dewax™	3 min	3	3
Pretreatment-Nucleic Acid Retrieval	2 mins at 85˚C and 20 mins at 98˚C	3	3
Prehybridization Buffer	30 mins at 37°C	0	0
Probe	2 hour at 37°C	0	3
Wash Solution A	5 mins at 45°C (2 cycle)	0	2
Wash Solution B	5 mins at 45°C (2 cycle)	0	2
Peroxide Block	10 mins at RT	0	2
Power Block	10 mins at RT	0	0
Anti fluorescein	30 mins at RT	0	3
Poly-HRP	30min	0	2
DAB	5-15 min	6	0
Hematoxylin	1 min	6	

Disclaimer: The above information is provided for reference only. Each end-user is responsible for developing and validating optimal testing conditions for use with this product.

Quality Control:
This product is quality control tested at BioGenex according to the suggested procedure. The recommended positive control tissue for this miRNA probe is cervical carcinoma.

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

Expected Results
Proper use of this probe will result in an intense stain at the specific site of the hybridized fluorescein-labeled probe in positive test tissue. If staining is absent from any positive control slides, or present in any negative control slides, the test should be considered invalid.

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.

Performance Characteristics
BioGenex has conducted studies to evaluate the performance of the probe with BioGenex detection systems and accessories. The probes have been found to be sensitive and show specific binding to the target nucleic acid of interest with minimal to no binding to non-specific tissues or cells. BioGenex probes have shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated methods. BioGenex ensures product quality through standard quality control for all products released and through surveillance programs.

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