BioGenex

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Hsa-miR-23A Probe

Catalog No. HM23A-100E
Description: one vial of 0.650 ml of probe in hybridization buffer

Intended Use:
This probe is intended for *in-vitro diagnostic use only.*

Principle:
MicroRNA's (miRNA's) are endogenous, non-coding small RNA molecules that play important role in controlling gene expression. They are found to engage in multiple biological function and disease progression including cancer. miRNA either acts as tumor suppressor or oncogene depending on function of their target gene. Aberrant expression of miRNA has been reported in different cancer types hence in-situ detection of miRNA provides important insight for diagnosis, prognosis and disease management. MiR-23a is known to play important role in the development of cancers and deregulated in various hematological malignancies and solid tumors. Patients with diffused large B-cell lymphomas have shown positive correlation with higher expression level of miR-23a and shorter survival rate than patients with a lower expression level. miR-23a, is shown to participate in invasion of pancreatic ductal adenocarcinoma cells into the mesothelial barrier and acts as oncogenic regulator for proliferation pancreatic cell and apoptosis by targeting APAF-1 (apoptotic protease activating factor 1) protein.[9] In hepatocellular carcinoma cells miR-23a has been observed to function as a growth-promoting and anti-apoptotic factor. In gastric adeno-carcinoma cells and tissues, upregulated miR-23a promoted aberrant cell proliferation activity by downregulating IL6 receptor expression.[10] While, in breast cancer it enhances disease progression by directly activating FOXM1 (Forkhead box protein M1) and HRG (Histidine-rich glycoprotein) at RNA level.These studies highlight the prognostic significance of miR-23a in clinical practice.

Please visit the following link for more information about hsa-miR-23a.http://www.ncbi.nlm.nih.gov/gene/407010

Summary and Explanation
miRNA's play an important role in many biological processes, including differentiation and development, cell signaling, and response to infection. Recent research have shown that human miRNA genes are frequently located in cancer-associated genomic regions, while perturbed miRNA expression patterns have been observed in many human cancers. A number of oncogenes and tumor suppressor genes were found to be targets of miRNA's and global miRNA expression signatures were able to distinguish between cancerous and non-cancerous tissues. The microRNA profiles can serve as highly specific markers for diagnosis, prognosis, disease monitoring as well as prediction of therapeutic response. miRNA's are remarkably stable molecule to be well preserved in FFPE as well as frozen specimens. Early diagnosis detection and assessment of the disease progression are essential for disease management, especially in tumor patients, where timely therapeutic interventions are extremely critical.

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 secondary 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
EZ-DeWax™ (Cat # HK585), SuperSensitive™ wash buffer (Cat # HK583), and Super Sensitive one-step Polymer-HRP ISH detection kit (Cat # DF400) need to be ordered separately. Please refer to the package insert(s) of detection kit for detailed protocols and instructions on use of the reagents.

Recommended detection system:
Super Sensitive One step Polymer-HRP ISH Detection Kit.

Storage and Handling
Store the probe at 2-8° 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.

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Treatment of Tissues Prior to Staining

All formalin-fixed, paraffin-embedded tissue sections require pretreatment with Nucleic Acid Retrieval 1 solution (NAR-1) Cat. No. HK873.

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 Super Sensitive one-step Polymer-HRP ISH detection kit (Cat# DF400) is used to detect hybridized probes following the instructions in the package insert for the detection system.

The negative and positive control probe is run in parallel.

Recommended protocol and parameters for Hsa-miR-23a probe

Table 1: Protocol for Manual Staining Procedure -FFPE tissue section

S.No	Reagents	Cat #	ITT	Cycle	No. of buffer wash
1	Baking	NA	20 min, 70°C		0
2	EZ-DeWax™	HK585	10 min, RT	3	0
3	100% Alcohol	NA	10 min, RT	2	2
4	NAR1 (Pretreat ment)	HK601	2 min, 85 °C 20 min, 98 °C	1	3
5	Pre-hybridization buffer	HK881	20 min, 42 °C	1	0
6	Probe-Hybridization (Hsa-miR-23a)	HM23A-100E	2 hrs, 42 °C	1	3
7	Wash A	HK839	5 min, 47 °C	2	2
8	Wash B	HK880	5 min, 52 °C	2	2
9	Peroxide Block	HK026	10 min, RT	1	2
10	Power Block	HK083	10 min, RT	1	0
11	Anti-Fluorescene AB	HK818	30 min, RT	1	3
12	Poly-HRP	HK943	40 min, RT	1	2
13	DAB	HK124	10 min, RT	1	3
14	Hematoxylin	HK030	1-3 min	1	3+3*

3*-DI WATER WASH

PS:

1. Supersensitive ISH detection kit(DF-400) is recommended for miRNA detection.
2. EZ-DeWax™ (Cat # HK585) and SuperSensitive™ wash buffer (Cat # HK583) are not part of kit component and need to be ordered separately.
3. Heat pretreatment with NAR1 can either be done at manual staining platform Xmatrx mini with BioGenex proprietary technique of oil sealing and coverslip or in the EZ-Retriver with bulk NAR1 reagent.
4. Coverslips should be used at NAR1, probe and antibody steps to achieve proper reagents spread and minimize the usage of reagent. This also ensures prevention of evaporation and drying during heating.

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 human GIST and reactive endomyometrium tissues (FB-HM023A).

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 and positive controls. If staining is absent from any positive control slides, or present in any negative control slides, the test should be considered invalid.

Nuclear and cytoplasmic staining pattern when tested in human GIST and reactive endomyometrium tissues were observed. It has been long assumed that mature miRNA can only be found in the cytoplasm but recent reports revealed the nuclear localization of mature miRNA. If tissue or cells are not processed optimally then you may see non-specific staining in connective tissue.

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

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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.

8°C 2°C	Temperature Limitation	IVD	In Vitro Diagnostic Medical Device
	Use By Date	LOT	Batch Code
NON STERILE	Non-Sterile		Consult Instructions for Use
REF	Catalogue Number		BioGenex

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