

Hsa-miR-376C Probe

Catalog No. HM376C-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:

MicroRNAs (miRNAs) 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-376c, is overexpressed in a subset of acute myeloid leukemia samples. It has also been shown that the miR-376 cluster transcripts can undergo adenosine-to-inosine editing in a tissue-specific manner, leading to the silencing of a different set of genes. Ye et al., have demonstrated that miR-376c targets ALK7 expression to promote proliferation and survival of ovarian cancer cells. Ye et al., have also provided evidence that the Nodal-ALK7 pathway and miR-376c are involved in cisplatin-induced cell death. Future *in vivo* studies will further clarify the role of miR-376c in ovarian cancer development and chemo-resistance. miR-376c expression level was decreased while transforming growth factor- α (TGFA) mRNA expression levels were increased in osteosarcoma tissues and cell lines, and Jin et al., identified TGFA as a novel direct target of miR-376c. Overexpression of miR-376c suppressed TGFA expression and the expression of its downstream signaling molecule such as epidermal growth factor receptor, and attenuated cell proliferation and invasion. Taken together, these findings will shed light on the role and mechanism of miR-376c in regulating osteosarcoma cell growth via miR-376c/TGFA axis, and miR-376c may serve as a potential therapeutic target in osteosarcoma in the future. Reciprocal antagonism between miR-376c and Smad signaling that regulates cell fate during human neural differentiation. In the undifferentiated state in hESCs, TGF- β -SMAD signaling represses neural differentiation by directly suppressing miR-376c and the neural factor Pax6. Inhibition of TGF- β -SMAD signaling releases miR-376c and Pax6 to trigger neural differentiation. Thus, Liu et al., model provides insight into how the signaling pathway and miRNAs are coordinated to regulate cell fate.

Please visit the following link for more information about hsa-miR-376c. <http://www.ncbi.nlm.nih.gov/gene/442913>

Summary and Explanation

miRNAs 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 miRNAs 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. miRNAs 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.

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

- The BioGenex miRNA probes are supplied in hybridization buffer and used without further dilution.
- The probe solution is brought to room temperature just prior to use.
- All testing is done at room temperature. The testing parameters and testing protocols are listed in Table 1 below.
- 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-376C 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 (Pretreatment)	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-376C)	HM376C-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-Fluorescence 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

Note:

- Supersensitive ISH detection kit(DF-400) is recommended for miRNA detection.
- EZ-DeWax™ (Cat # HK585) and SuperSensitive™ wash buffer (Cat # HK583) are not part of kit component and need to be ordered separately.

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- 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-Retraver with bulk NAR1 reagent.
- 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 bone tissues(FB-HM376C).

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 staining pattern when tested in human Bone and tissues was 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.

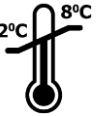
Performance Characteristics

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 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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	Temperature Limitation		In Vitro Diagnostic Medical Device
	Use By Date		Batch Code
	Non-Sterile		Consult Instructions for Use
	Representative in the European Community		Manufacturer