# hsa-miR-21-5p miREIA kit





- > Typical onco-miRNA
- > Upregulated in solid tumors
- > Cardiovascular disease
- > Pulmonary diseases
- > Immunological and developmental processes

# <sup>5'-UAGCUUAUCAGACUGAUGUUGA-3,</sup>



# > Circulating miRNA

- > One of the first identified
- > One of the most frequently studied
- > Strongly conserved throughout evolution

# *miREIA* (*miR*NA *E*nzyme *I*mmuno*a*ssay)

- · Quantitative method
- · MAb specific to miRNA/DNA probe
- · ELISA platform
- · Patented!
- · NEW!



#### **Function of microRNAs**

MicroRNAs (miRNAs) are small non-coding RNA molecules, approximately 22 nucleotides in length that regulate gene translation through silencing or degradation of target mRNAs. They are involved in multiple biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation, and in pathophysiology of many diseases. Numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of many diseases.

#### miR-21-5p – The most frequently studied microRNA

miR-21-5p stands out as the most representative miRNA biomarker as it belongs to the first identified miRNA molecules. miR-21-5p is a circulating miRNA, strongly conserved through evolution, which has been extensively explored in a range of studies on various diseases, such as cancer, cardiovascular disease, pulmonary diseases, regulation of immunological processes, and many others.

#### miR-21-5p – A promising biomarker in the diagnosis and prognosis of various cancers

miR-21-5p was identified as a typical onco-miRNA dysregulated in most cancers by acting as an oncogene. Besides in tissues, recent evidence indicates the presence of miR-21-5p in many types of extracellular fluid, such as plasma, serum, CSF, saliva, gastric juice, pancreatic juice, sputum, and pancreatic cyst fluid. Up-regulated miR-21-5p could increase tumor growth, metastasis and invasion and reduce sensitivity to chemotherapy by its multiple targets. The evidence from published metaanalyses revealed that high expression level of miR-21-5p was a negative predictor for survivals in various cancers.



#### miREIA (miRNA Enzyme Immunoassay) - a novel, immunoassay-based method of miRNA quantification

miRNA isolated from a patient sample is hybridized to complementary biotinylated DNA oligonucleotide probe.

The DNA/RNA hybrids are then processed in the manner of ELISA in a microtiter plate coated with a monoclonal antibody specific to perfectly matched DNA/miRNA hybrids.

After washing, the solid phase is incubated with streptavidin-HRP conjugate and the resulting complexes are visualized (after another washing step) by a chromogenic substrate.

The miREIA exhibits superior analytical specificity, limit of detection as low as 0.1 attomol/ $\mu$ l miRNA, excellent analytical characteristics and strong correlation with the qRT-PCR method (Pearson correlation coefficient >0.9).

miREIA can be run on common immunoassay analyzers, is compatible with standard clinical workflow, does not require amplification steps and results are obtained in less than three hours including miRNA profiling.

## miR-21-5p – The mostly investigated cancers

The mostly investigated cancers where miR-21-5p was explored included brain cancer, lung cancer, colorectal cancer, pancreatic cancer, breast cancer, gastric cancer, esophageal cancer and hepatocellular carcinoma. Among them:

**Pancreatic cancer:** miR-21-5p was found to be a highly prevalent in serum samples for PC patients. The diagnostic accuracy of serum miR-21-5p was found to be relatively high and circulating hsa-miR-21-5p might be a promising serum biomarker in patients with PC.

**Colorectal cancer:** circulating miR-21-5p was described as a suitable diagnostic biomarker, tissue miR-21-5p as a prognostic marker.

**Gastric cancer:** it has been demonstrated that the expression levels of miR-21-5p were reduced following surgical removal of gastric cancer tissues and urine miR-21-5p could be utilized as a novel non-invasive biomarker of gastric cancer detection and monitoring.

# miR-21-5p – In cardiovascular disease

miR-21-5p is highly expressed in the cardiovascular system. Recent studies have revealed that its expression is dysregulated in the heart under cardiovascular disease conditions such as proliferative vascular disease, cardiac hypertrophy and heart failure or ischemic heart disease. miR-21-5p has been found to play important roles in vascular smooth muscle cell proliferation and apoptosis, cardiac cell growth and death, calcific aortic valve disease and cardiac fibroblast functions. Plasma miR-21-5p has been described as a proposed novel biomarker of acute myocardial infarction exhibiting similar diagnostic accuracy with traditional markers including CK, CKMB and cTnI.



Comparison of miREIA to RT-qPCR				
	miREIA	qRT-PCR		
Reverse transcription reaction	NOT required	Required		
Amplification steps	NOT required	Required		
Total time to result	2 hours	> 3 hours		
Quantification	Absolute (attomol/µl)	Relative		
Instrumentation required	Immunoassay equipment	Specific PCR cyclers		
Cost per sample	Lower	Higher		

### **Analytical characteristics**

Limit of detection	0.1 attomol/µl
Dilution linearity	82 - 116 %
Spiking recovery	96 - 123 %
Intra-assay CV	3 - 9 %
Inter-assay CV	3.5 - 15 %



### **Specificity**

Cross-reactivity with other 22 miRNAs have been less than 0.01 % (negligible signal at 1000 attomol/ $\mu l$ ).

The following miRNAs were tested:

miR-15a-5p	miR-16-5p	miR-23a-3p	miR-28-3p
miR-24-3p	miR-27a-3p	miR-27b-3p	miR-93-5p
miR-122-5p	miR-126-3p	miR-126-5p	miR-142-5p
miR-146a-5p	miR-150-5p	miR-191-5p	miR-197-3p
miR-221-5p	miR-222-3p	miR-223-3p	miR-320a
miR-376c-3p	miR-451a		



microRNA-21 isolated from whole blood taken from 10 healthy volunteers was quantified by miREIA-21-5p and by qRT-PCR using LNA nucleotide probes from Exiqon.

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