

**BioVendor
Group**

miRNA

ENG

Manual for

Two-Tailed qPCR Assays

For research use only!

 **BioVendor
R&D[®]**

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

1.	DESCRIPTION	3
2.	STORAGE, EXPIRATION	3
3.	ASSAY PRINCIPLE	4
4.	RECOMMENDED MATERIAL	4
5.	ASSAY PROCEDURE	5
6.	FREQUENTLY ASKED QUESTIONS	6
7.	EXPLANATION OF SYMBOLS	7

Example Version

1. DESCRIPTION

This product data sheet is valid for all available Two-Tailed qPCR Assays.

TT-qPCR assays are designed for detection and accurate quantification of microRNA targets with real-time cyclers capable to detect at SYBR/FAM channel and which do not require using of ROX as passive reference dye.

For use in qPCR instruments enabling the use of ROX dye it is necessary to set the *Passive reference to none*.

The kits are suitable for detection of miRNAs isolated from different biological samples, including serum, plasma, blood, urine, tissues etc.

The users are supposed to choose an appropriate kit for RNA isolation themselves, depending on the sample type. For RNA isolation, use e.g. BioVendor RNA Isolation Kits: www.biovendor.com/mirna-isolation-kits

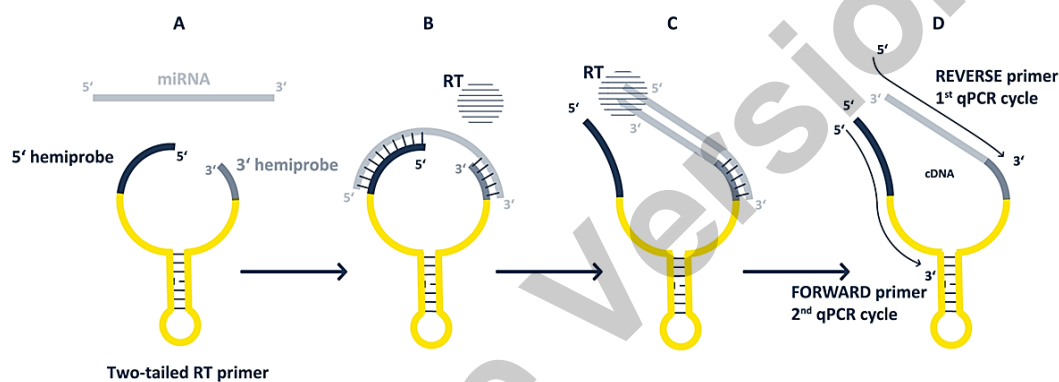
2. STORAGE, EXPIRATION

The kits are to be transported and stored at temperatures ranging from -15 °C to -70 °C. Temperatures above this range may adversely affect the performance of this kit.

The kits remain stable for 12 months from the date of manufacturing at the temperature of -20 °C. The components of the kit are stable for 6 months at -18 °C to -25 °C after the first opening.

3. ASSAY PRINCIPLE

Two-Tailed qPCR uses one Two-tailed RT primer which consists of two hemiprobcs, 3'-hemiprobe and 5'-hemiprobe connected by a folded tether (A). The Two-tailed RT primer binds to different stretches of the microRNA (B). While each hemiprobe is too short to bind the microRNA, when both hemiprobcs are complementary to their target miRNA, they bind cooperatively and specifically. Binding is exceeding specific, as a mismatch is much more profound in a short hemiprobe. The cDNA formed after Reverse Transcription (C) can then be PCR amplified using two sequence specific PCR primers (D). **SYBR fluorescent dye is used for the detection.** High melting resolution analysis can be used for non-specific products detection.



4. RECOMMENDED MATERIAL

Each miRNA assay consists of three parts:

Name of product	Catalogue number
miR-TT-PRI (set of specific primers: 1 x RT primer, 2 x PCR primer)	RDTT(MIMAT number)PRI
Two-Tailed cDNA Synthesis System 50 rxn (RT)	RDTTRT50
Two-Tailed qPCR Master Mix 150 rxn (qPCR)	RDTTPCR150

5. ASSAY PROCEDURE

5.1 REVERSE TRANSCRIPTION

It is necessary to use RT primer from the miR-TT-PRI kit containing set of specific primers and the Two-Tailed cDNA Synthesis System 50 rxn, Cat. No RDTTRT50, which contains RT mix (10x), RT Enzyme and Nuclease Free Water.

- 1) Thaw and mix the following components for 1 reaction. Keep RT Enzyme on ice! Avoid vortexing of RT Enzyme.

Component	Volume
RT Mix (10x)	2.00 μ l
Nuclease Free Water	11.75 μ l
RT primer	0.25 μ l
RT Enzyme	2.00 μ l
RNA (1 μ g – 10 pg)	4.00 μ l
TOTAL	20.00 μ l

- 2) Perform the Reverse Transcription on thermocycler or thermoblock according to the following protocol:

Step	Temperature	Time
1	25 °C	5 min
2	50 °C	15 min
3	85 °C	5 min
4	4 °C	infinite hold

- 3) Dilute the resulting cDNA by adding 80 μ l nuclease free water. Undiluted cDNA can be stored at -20 °C for up to 4 weeks. Please avoid repeated freeze-thaw cycles.

5.2 QUANTITATIVE qPCR AMPLIFICATION AND DETECTION

It is necessary to use PCR primers (PCR Primer F and PCR Primer R) from the miR-TT-PRI kit containing set of specific primers and the Two-Tailed qPCR Master Mix 150 rxn kit, Cat. No RDTTPCR150, which contains PCR mix (2x) and Nuclease Free Water.

Quantitative qPCR is done with individual reactions for each miRNA or sncRNA target. No multiplexing is possible! Triplicates of PCR reaction are recommended to each sample of every target.

1) Thaw and mix the following components for 1 reaction:

Component	Volume
PCR Mix (2x)	10.0 μ l
Nuclease Free Water	5.2 μ l
PCR Primer F	0.4 μ l
PCR Primer R	0.4 μ l
cDNA	4.0 μ l
TOTAL	20.0 μ l

2) Perform polymerase chain reaction according to the following protocol:

Step	Temperature	Time
1	95 °C	30 s
2	95 °C	5 s
3	60 °C	15 s
4	72 °C	10 s (SYBR/FAM)
5	GO TO 2	40x repetition
6	Melting curve (SYBR)	





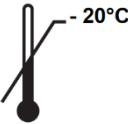


3) Analyse results (Ct and Melting temperature) according to your thermocycler software.

6. FREQUENTLY ASKED QUESTIONS

Frequently asked questions are available on our website:

<https://www.biovendor.com/two-tailed-rt-qpcr>

7. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
 www.biovendor.com	Read electronic instructions for use - eIFU



BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

