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

CONTENTS

## 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

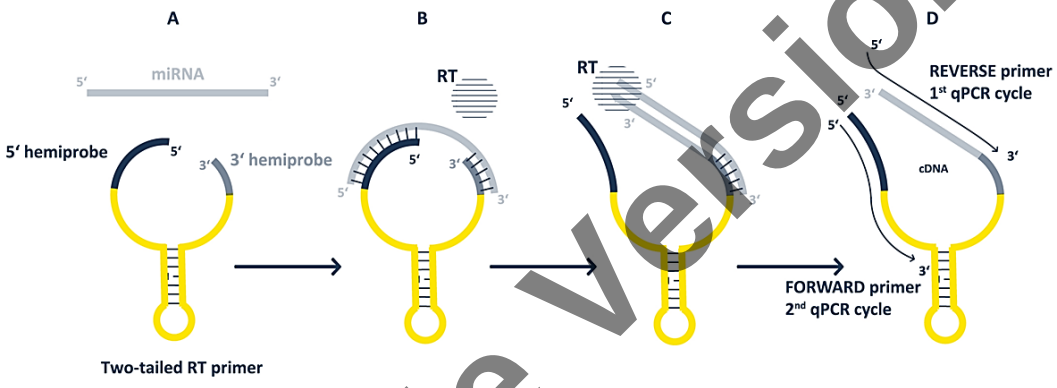
The kits are to be transported and stored at temperatures ranging from -15 °C to -70 °C. Temperatures above this range may adversely affect the performance of this kit.

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 hemiprobes,

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 hemiprobes 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<br>(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 REVERESE 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 µl |
| Nuclease Free Water | 11.75 µl |
| RT primer | 0.25 µl |
| RT Enzyme | 2.00 µl |
| RNA (1µg – 10 pg) | 4.00 µl |
| TOTAL | 20.00 µl |

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 µl 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 µl |
| Nuclease Free Water | 5.2 µl |
| PCR Primer F | 0.4 µl |
| PCR Primer R | 0.4 µl |
| cDNA | 4.0 µl |
| TOTAL | 20.0 µl |

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) | |



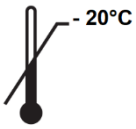
3) Analyse results (Ct and Melting temperature) according to your thermocycler software.

# 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 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