BioVendor Group miRNA

ENG

Manual for

Two-Tailed qPCR Assays

For research use only

BioVendor R&D®

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

CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.003.A	ENG.004.A
In Chapter 2, the kit's expiration date has changed.	
In Chapter 6, FAQs have been added.	
In Chapter 7, the caution symbol was removed.	
Change in the company's legal form.	

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 18 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**. Standard melt curve analysis can be used for non-specific products detection.

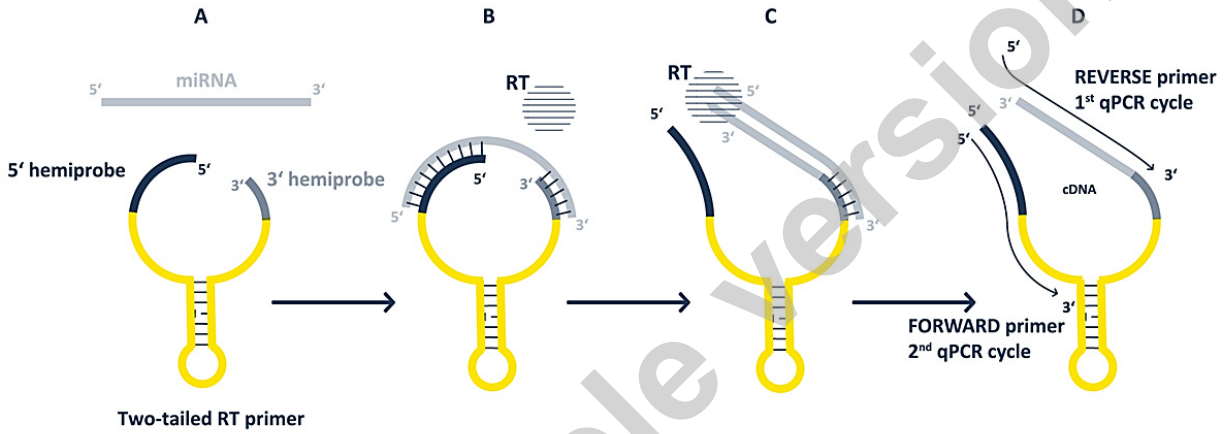


Figure 1: Principle of TT-qPCR assay.

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 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. FAQ

1. What types of molecules can be quantified by Two-Tailed qPCR?

Our patented TT-qPCR platform has superior performance in the detection and quantification of short sequence RNA molecules (<300bp). This includes short and mid-sized non-coding RNAs (sncRNAs & mncRNAs) like microRNAs, Piwi-interacting RNAs, snoRNAs and others. In various diagnostic panels, it is possible to combine more types of targets, e.g., piRNAs and miRNAs.

2. How many samples does one Two-Tailed PRIMER kit measure?

The basic TT-PRIMER kit includes reverse transcription (RT) primer for 50 samples and two qPCR primers (forward and reverse) for 150 reactions as we believe in golden standard of running PCR in a triplicate per sample.

3. Which products shall I purchase to run this assay?

Complete TT-qPCR assay includes Two-Tailed PRIMERs (RDTT000****PRI), Two-Tailed cDNA Synthesis System 50rxn (RDTTRT50) and Two-Tailed qPCR Master Mix 150rxn (RDTTPCR150). There is also a possibility to order a synthetic miRNA (TTK0000****) with various applications, e.g., isolation spike-in control, positive control, absolute quantification etc.

4. Can I multiplex several targets in one reaction?

Yes, there is a possibility to multiplex up to 5 targets within one RT reaction. This approach is very sequence dependent, and our specialists perform laboratory tests to verify there is no cross reactivity or alterations in assays characteristics like sensitivity or specificity. Following PCR reaction always runs separately for each target as the detection is through SYBR Green dye.

5. What laboratory equipment is necessary to run the Two-Tailed qPCR?

Our assays can be run in any laboratory equipped for standard qPCR measurement. You need a vortex mixer and benchtop centrifuge, standard block heater or thermocycler and Real-Time PCR system with FAM/SYBR Green detection channel, e.g. CFX96 Real-Time PCR Detection System (Bio-Rad).

6. **Could I order primers for ANY microRNA targets?**

   YES, besides already developed assays (list of them at www.biovendor.com/two-tailed-rt-qpcr) we offer also customized development and production of TT-PRIMERs. So whatever miRNA, piRNA, siRNA, SNORD or other sncRNA/mncRNA you are interested in, we will develop a unique, specific, and highly sensitive assay to quantify it. Development time is approximately 6-8 weeks. It can be altered in case of additional testing, e.g., for multiplex reaction.

7. **How is a TT-qPCR assay developed?**

   Our team of specialists always starts with in-silico design of all primers using a comprehensive algorithm. Our unique Two-Tailed RT primers are then laboratory tested together in several combinations with various qPCR primers to identify the optimal combination of RT and qPCR primers which meets all our requirements for an assay performance. That means that we always run various laboratory tests to make sure that the assays quantify only desired targets. All laboratory tests were performed using a CFX96 Real-Time PCR Detection System (Bio-Rad).

8. **What type of controls and normalisation shall I use for my project?**

   There are several options how to properly approach normalisation and what controls to include in your research. Regarding controls, besides including the blank negative control of RT reaction and PCR reaction, we suggest usage of a positive control (synthetic miRNA molecule – available at www.biovendor.com/two-tailed-rt-qpcr). It is always wise to control very important step, the isolation of RNA. Additionally, we prepared an isolation control kit which contains an artificial miRNA in the combination with a specific C.elegans miRNA. These two spike-ins are used to monitor the isolation efficiency. This can be further used also as an exogenous normalization and mostly it works as a comparison between each sample which enters the TT-qPCR protocol. You can also choose a stable expressed sncRNA target known to be present in your particular sample type which works as an endogenous normalization control. Last option is to prepare a dilution set of a synthetic miRNA of your choice and measure it as a calibration curve for an absolute quantification of your target.

9. What isolation kits shall I use to obtain ideal performance?

There are many producers available on the market and it is always dependent on your experiences but, our assays are tested and validated with RNA isolates eluted from our own isolation kits (www.biovendor.com/mirna-isolation-kits). They are all column-based, sample type specific, user friendly with very attractive and competitive price. Our specialists are always willing to provide a consultation, or any troubleshooting based on your individual project settings. When you are planning your project, it is very important to use the same isolation procedure across all your experiments you would like to compare. Be aware of using and combining samples proceeded by different isolation kits.

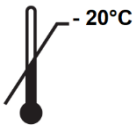
10. What sample types can be measured by TT-qPCR assay?

TT-qPCR assay can measure RNA isolated from any type of biological material (tissues, cell lines, solid tumours, etc.) and biofluids (blood, serum, plasma, urine, sweat, etc.). The detection of the specific target in your biological sample depends on its concentration in the sample, so we recommend testing the capture in the small preliminary study. In the case of customised assay development, our specialist can test the capture in your specific samples during the development of the assay.

11. How long does it take to run a TT-qPCR assay?

Starting with already isolated RNA from your samples, you need to prepare reverse transcription mixture, add your isolates, and run the RT reaction. This is always number of samples dependent, but it can be done within 1 hour in total, including the further dilution of your cDNA products. Following step is qPCR measurement. Once you prepare the Master Mix, you need to pipette it onto your PCR plate and add cDNA from your samples. This can be managed within 30 minutes with automatic pipette and then you need to run the reaction program which takes additional 60 minutes. With proper equipment, you can run Two-Tailed qPCR in only 2.5 hours.

7. EXPLANATION OF SYMBOLS

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

BioVendor R&D®

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