

Manual for TT-RT-qPCR kits

Description

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

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

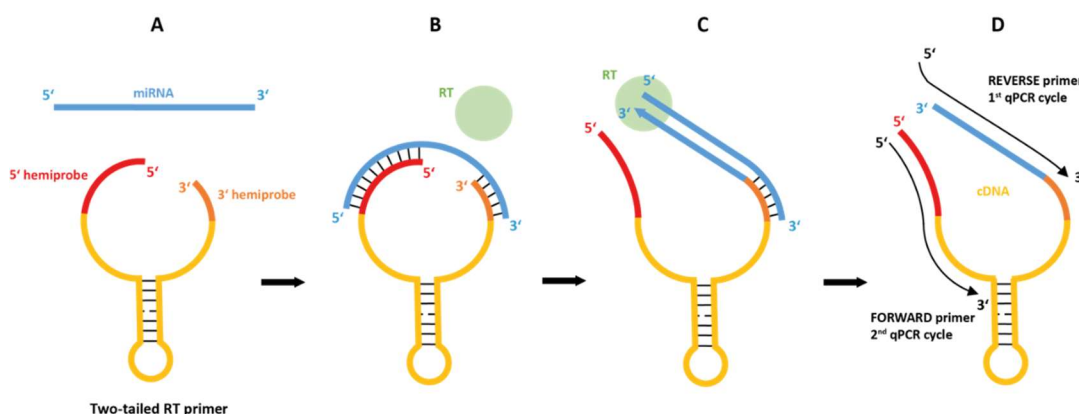
The recommended isolation kits and parameters of miRNA isolates quality are available here: <https://www.biovendor.com/mirna-isolation-kits>

Storage and Expiration

Kit components 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 kit remains 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.

Assay principle

Two-tailed PCR uses two hemiprobosc, which bind to different stretches of the microRNA, that are connected by a folded tether. While each hemiprobe is too short to bind the microRNA, when both hemiprobosc are complementary they bind cooperatively. Binding is exceeding specific, as a mismatch is much more profound in a short hemiprobe. The cDNA formed can then be PCR amplified using two sequence specific primers. SYBR used for detection. High melting resolution analysis can be used for non-specific products detection.



Each miRNA assay consists of two parts which can be ordered separately:

- 1) Reverse transcription of specific miRNA
- 2) Amplification of specific miRNA by real-time PCR followed by melting temperature analysis

More details and technical support: <https://www.biovendor.com/two-tailed-qpcr>

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Reverse transcription

- 1) Thaw and Mix the following components for 1 reaction

RT Mix	2,00 µl
Nuclease free water	11,75 µl
RT primer	0,25 µl
Enzyme	2,00 µl
RNA (1µg – 10 pg)	4,00 µl
TOTAL	20,00 µl

- 2) Perform reverse transcription according the following protocol

STEP	TEMPERATURE	TIME
1	25 °C	5 min
2	50 °C	15 min
3	85 °C	5 min
4	4 °C	forever

- 3) Dilute the resulting cDNA by 80 µl nuclease free water

*Undiluted cDNA can be stored at -20 °C for up to 4 weeks. Please avoid repeated freeze-thaw cycles

Real-time qPCR amplification and detection

- 1) Thaw and Mix the following components for 1 reaction

PCR Mix	10,0 µl
Nuclease free water	5,2 µl
Primer F	0,4 µl
Primer R	0,4 µl
cDNA	4,0 µl
TOTAL	20,0 µl

- 2) Perform polymerase chain reaction according 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 (collect data SYBR/FAM)
5	GO TO 2	40x repetition
6	Melting curve (SYBR)	

- 3) Analyse results according your thermocycler software

More details and technical support: <https://www.biovendor.com/two-tailed-qpcr>