BioVendor R&D®

Manual for TT-qPCR assay

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.

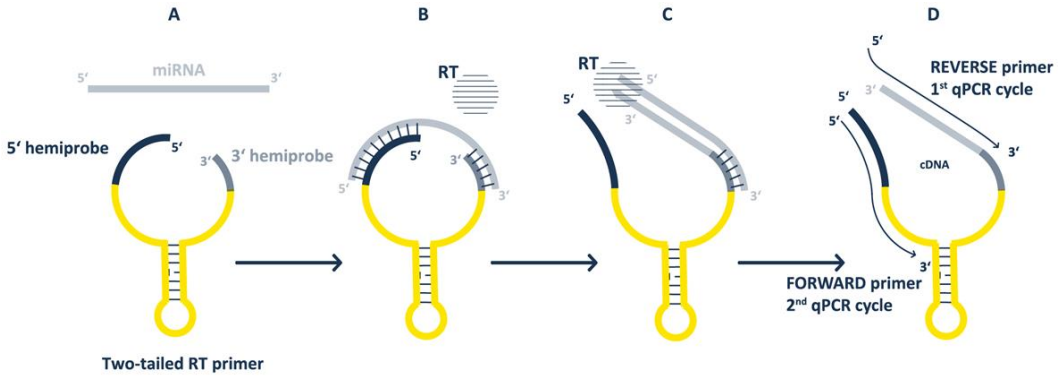
The recommended isolation kits and parameters of miRNA isolates quality are available here: https://www.biovendor.com/mirna-isolation-kits

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 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.



Each miRNA assay consists of three parts:

1) Set of specific primers (1 x RT, 2 x PCR)
2) cDNA synthesis system (RT)
3) qPCR Master Mix

Edited: 30.04.2021

BioVendor – Laboratorni medicina a.s.
Karasek 1767/1
621 00 Brno
Czech republic

Company ID: 634 71 507 | VAT No: CZ63471507
Registered in the Commercial Register maintained
by the Regional Court in Brno, section B, file 3917

TEL: +420 549 124 111
FAX: +420 549 211 465
info@biovendor.com
www.biovendor.com

BioVendor R&D®

Manual for TT-qPCR assay

Reverse Transcription

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 to 80 µl nuclease free water. Undiluted cDNA can be stored at -20 °C for up to 4 weeks. Please avoid repeated freeze-thaw cycles.

Edited: 30.04.2021

BioVendor – Laboratorni medicina a.s.
Karasek 1767/1
621 00 Brno
Czech republic

Company ID: 634 71 507 | VAT No: CZ63471507
Registered in the Commercial Register maintained
by the Regional Court in Brno, section B, file 3917

TEL: +420 549 124 111
FAX: +420 549 211 465
info@biovendor.com
www.biovendor.com

BioVendor R&D®

Manual for TT-qPCR assay

Quantitative qPCR amplification and detection

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.

Edited: 30.04.2021

BioVendor – Laboratorni medicina a.s.
Karasek 1767/1
621 00 Brno
Czech republic

Company ID: 634 71 507 | VAT No: CZ63471507
Registered in the Commercial Register maintained
by the Regional Court in Brno, section B, file 3917

TEL: +420 549 124 111
FAX: +420 549 211 465
info@biovendor.com
www.biovendor.com