Taqman QRT Kit

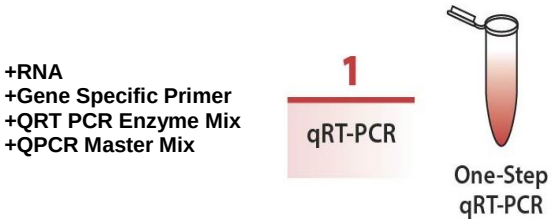
(Cat# M1183-100; One Step Taqman QRT PCR Kit; No Dye; Store at -20°C)

I. Introduction:

Taqman QRT Kit is a complete one step QPCR system. This QRT PCR Kit contains all reagents necessary for both Reverse Transcription(RT) and Taqman Probe based QPCR amplification to occur in a single QPCR reaction tube. The One-Step Taqman QRT PCR kit is an amalgamation of two key formulations; the QRT PCR Enzyme Mix and the Taqman 2X QRT PCR Mastermix within a proprietary blend of stabilizers and enhancers to enable a seamless coupling of two separate reactions into a real-time "single step" procedure. One-Step Taqman QRT PCR Kit uses a combination of high-quality enzymes in a proprietary buffer System to deliver precise and accurate sample analysis for high-throughput applications. This kit offers ultimate convenience in addition to consistent performance in terms of high sensitivity and superb signal-to noise ratio. While a one-step/single tube setup provides overall convenience and reduces room for error, BioVision's One-Step Taqman QRT PCR Kit offers additional advantages:

- Improved fidelity and yield for reverse transcription
- Prevention of template (RNA) degradation with RNaseOFF Ribonuclease Inhibitor.
- Superb performance with respect to sensitivity and signal-to-noise ratio.
- Significant reduction in non-specific PCR amplification by utilizing HotStart Taq DNA polymerase in the enzyme mix.

BioVision's Taqman QRT PCR Kit offers convenient real-time RNA quantification in one EASY step. Please refer to our QPCR Master Mix Selection Guide for selecting the appropriate QPCR formulation applicable to your particular instrument model.



II. Application:

- Gene-expression analysis
- Transcription analysis
- Gene cloning
- High throughput applications
- Virus detection and quantification

III. Key Features:

- Streamlined protocol in a simple single-tube reaction set-up
- High-quality, full-length cDNA from as little as 0.01 pg of RNA
- Fully optimized for detection of low-copy genes
- Simple set-up for any RNA template
- Reduces pipetting steps to minimize the risk of contamination

IV. Package Contents (Taqman One Step QRT PCR Kit):

Components	M1183-100 (100 X 20 µl rxns)	Part Number
Taqman QRT PCR Master Mix-No Dye	1.25 ml	M1183-XX-1
QRT PCR Enzyme Mix (50X)	40 µl	M1183-XX-2
Nuclease-free H_2O	1 ml	M1183-XX-3

V. User Supplied Reagents and Equipment:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Total RNA or poly(A) + mRNA
- Taqman Probe

VI. Shipment and Storage:

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly. Avoid repeated freeze-thaw cycles to retain maximum performance. Briefly centrifuge small vials prior to opening.

VII. Protocol:

RT PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR, and subsequent reaction analysis should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Prepare the following reaction mixture in a PCR tube on ice.

Components	Reaction Volume			Concentration
	10 µl	20 µl	50 µl	
Total RNA or poly(A) + mRNA	Variable	Variable	Variable	5 pg - 1 µg/rxn 0.05 pg - 20 ng/rxn
Taqman 2X QRT PCR Master Mix-No Dye	5 µl	10 µl	25 µl	1 X
QRT PCR Enzyme Mix (50X)	0.2 µl	0.4 µl	1 µl	1 X

Forward Primer (6 µM)	0.5 µl	1 µl	2.5 µl	300 nM
Reverse Primer (6 µM)	0.5 µl	1 µl	2.5 µl	300 nM
Taqman Probe	Variable	Variable	Variable	100 - 300 nM
Nuclease-free H_2O	Up to 10 µl	Up to 10 µl	Up to 50 µl	-

Notes: Gene specific primers must be used.

2. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
3. Program the thermal cycler so that cDNA synthesis is followed immediately by QPCR amplification.

Steps	Temperature	Duration	Cycle (s)
cDNA Synthesis	42°C	15 min	1
Pre-Denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing	60°C	60 sec	
Melt Curve	According to the instrumental guidelines		

VII. General Notes:

- Aliquot reagents to avoid risk of contamination and repeated freeze-thaw cycles.
- Avoid high primer concentrations; a concentration of 100 nM to 300 nM of each primer usually gives the best results.
- Probe concentration may require optimization, typically a 3:1 to 1:1 primer:probe ratio will give good amplification.
- A very effective way to get tight Ct among replicates is to reduce pipetting error, this can be achieved by: 1) preparing amplicon specific pre-mix, 2) using repeating pipettes and 3) keeping the pipetting volume within the manufacturer's suggested range.
- For optimal results, it is recommended that the primers are 18-22 nucleotides in length with a Tm of 58°C-60°C and the size of target is about 100-250 bp.

IX. Related Products:

BV Product Name	BV Cat. No.
Two Step RT PCR Kits	M1160-M1161
One Step RT PCR Kits	M1162-M1163
First Strand cDNA Synthesis Kits	M1164-M1167
First Strand cDNA Synthesis Supermixes	M1167-M1169
All-In-One RT Mastermixes	M1170-M1172
Reverse Transcriptases	M1173-M1174
One Step Jade™ QRT PCR Kits	M1175-M1182
One Step Taqman QRT PCR Kits	M1183-M1190

FOR RESEARCH USE ONLY! Not to be used on humans.