

Taqman QRT Kit-ROX

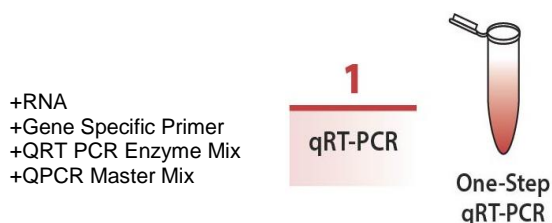
(Cat# M1186-100; One Step Taqman QRT PCR Kit; Store at -20°C)

I. Introduction:

Taqman QRT Kit-ROX is a complete one step QRT PCR system. This QRT PCR Kit contains all the 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):

Product Name	M1186-100 (100 X 20 µl rxns)	Part Number
Taqman QRT PCR Master Mix-ROX	1.25 ml	M1186-XX-1
QRT PCR Enzyme Mix (50X)	40 µl	M1186-XX-2
Nuclease-free H ₂ O	1 ml	M1186-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-ROX	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 μ M)	0.5 μ l	1 μ l	2.5 μ l	300 nM
Reverse Primer (6 μ M)	0.5 μ l	1 μ l	2.5 μ l	300 nM
Taqman Probe	Variable	Variable	Variable	100 - 300 nM
Nuclease-free H ₂ O	Up to 10 μ l	Up to 10 μ l	Up to 50 μ l	-

Notes: Gene specific primers must be used.

- Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
- 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 contamination and repeated freeze-thaw cycles.
- Taqman QPCR Master Mix components are light sensitive; avoid prolonged exposure to intense light.
- Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to QRT PCR reaction.

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.