Jade™ QRT Kit-ROX

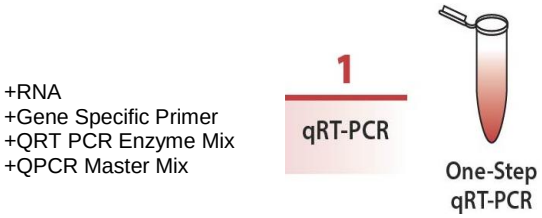
(Cat# M1178-100; One Step QRT PCR Kit; Store at -20°C)

I. Introduction:

Jade™ QRT Kit-ROX is a One-Step complete QRT PCR system containing all the necessary reagents for both reverse transcription and PCR amplification to occur in a single QPCR reaction tube. Specifically, this One-Step QRT PCR kit contains a QRT PCR Enzyme Mix and a Jade QPCR Master Mix. Our proprietary QRT PCR Enzyme Mix contains stabilizers and enhancers to optimize the two reactions in a real-time "single step". This One-Step QRT PCR kit offers the end-users an efficient, easy to use and reliable alternative to the conventional "twostep" sequential QRT PCR. Gene-specific primers must be used along with this kit.

The One-Step Jade QRT Kit utilizes a mutated recombinant M-MuLV Reverse Transcriptase which exhibits limited RNase H activity. RNaseOFF Ribonuclease Inhibitor and a proprietary RTase additive are blended into the RTase's storage buffer to protect RNA template from degradation and to improve the fidelity during reverse transcription. The chemically modified Hotstart Taq polymerase, included in our Mastermix, significantly reduces non-specific PCR amplification observed with regular Taq polymerase. The user-friendly one-step/single tube setup minimizes the chance of introducing human-error and contamination. Overall, BioVision's One-Step Jade QRT PCR Kit demonstrates market-leading sensitivity, efficiency, and reliability.

BioVision's Jade™ One Step RT PCR Kit offers a Convenient real-time RNA quantification in one-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
- Multiplex RT PCR
- 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 (Jade™ One Step QRT PCR Kit):

Components	M1178-100 (100 X 20 µl rxns)	Part Number
Jade™ Master Mix-ROX	1.25 ml	M1178-XX-1
QRT PCR Enzyme Mix (50X)	40 µl	M1178-XX-2
Nuclease-free H_2O	1 ml	M1178-XX-3

V. User Supplied Reagents and Equipment:

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

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	2 pg – 0.2 µg/rxn 0.01 pg - 2 ng/rxn
Jade™ 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 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
Nuclease-free H_2O	Up to 10 µl	Up to 10 µl	Up to 50 µl	-

Notes:

1. Gene specific primers must be used.
2. Amplicon should be <150 bp in size.
3. Please ensure no salt crystals are present in the Jade™ 2X QPCR Master Mix before use. If salt crystals are observed, mix until crystals are completely dissolved and absent.
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 contamination and repeated freeze-thaw cycles.
- Jade™ 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

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