Org Taqman QRT Kit

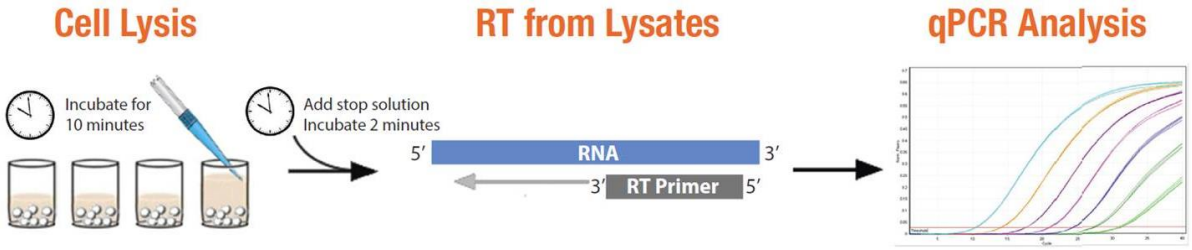
(Cat# M1187-100; One Step RNA Extraction from Cells and Taqman QRT PCR Kit; No Dye; Store at -20°C)

I. Introduction:

Org Taqman QRT Kit offers a quick, simple and robust method to prepare template for performing Taqman Probe based quantitative real-time analysis directly from cultured cells, enabling reverse transcription of lysates from 10-10^5 cultured cells without time-consuming and hazardous-chemicals-involved RNA extraction and purification steps. The kit includes reagents for cell lysis as well as gDNA removal. The lysis procedure simultaneously eliminates genomic DNA effectively in 12 min, without compromising RNA quality. The lysate can then be directly applied as template for One-Step QRT PCR, using the One Step Taqman QRT PCR Mastermix.

This One-Step Taqman QRT PCR system contains all necessary reagents for both reverse transcription and Taqman Probe based QPCR amplification to occur in a single qPCR reaction tube, including a QRT PCR Enzyme Mix in a proprietary buffer system to deliver precise and accurate sample analysis with high sensitivity and superb signal-to-noise ratio and an Taqman 2X QRT PCR Mastermix. The use of HotStart Taq DNA Polymerase in the enzyme mix significantly reduces non-specific PCR amplification. Our proprietary QRT PCR Enzyme Mix contains stabilizers and enhancers to optimize the two reactions in a real-time "single step". Coupled together, this complete system provides the ultimate convenience in generating consistent, reproducible, and accurate results from 10-10^5 cells.

BioVision's Org Taqman QRT PCR Kit offers Extraction and preparing RNA templates directly from cultured cells to be applied as template for One-Step QRT PCR. 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 studies

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

Components	M1187-100 (25 lysis preps, 100 X 20 µl rxns)	Part Number
Lysis Solution	1.25 ml X 2	M1187-XX-1
Stop Solution	300 µl	M1187-XX-2
Protease	50 µl	M1187-XX-3
Protease Inhibitor	50 µl	M1187-XX-4
Taqman Master Mix-No Dye	1.25 ml	M1187-XX-5
QRT PCR Enzyme Mix (50X)	40 µl	M1187-XX-6
Nuclease-free H_2O	1 ml	M1187-XX-7

IV. User Supplied Reagents and Equipment:

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

V. 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.

VI. Protocol:

1. Thaw Lysis Solution and Stop Solution. Homogenize each solution gently but thoroughly.
2. Prepare the following reactions for cell lysis:

Components	Volume
10-10^5 cells	5 µl
Protease	1 µl
Lysis Solution	50 µl

Mix content by pipetting 35 µl of the mixture up and down 5 times and avoid creating bubbles. Incubate at 37°C for 10 min, then add the following to the tube:

Protease Inhibitor	1 µl
Stop Solution	5 µl

Mix content by pipetting 35 µl of the mixture up and down 5 times. Incubate at room temperature for 2 min, then store the lysate on ice.

3. The lysate is ready for QRT PCR setup. Prepare the following reaction mixture in a QPCR tube on ice:

Components	Reaction Volume			Concentration
	10 µl	20 µl	50 µl	
Lysate from previous step*	1 µl	2 µl	5 µl	-

Taqman 2X QRT PCR Master Mix-No Dye	5 µl	10 µl	25 µl	1X
QRT PCR Enzyme Mix (50X)	0.2 µl	0.4 µl	1 µl	1X
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 20 µl	Up to 50 µl	-

Note:

1. As lysate is PCR-inhibitory in nature, the recommended volume of lysate used is 2%-10% of the reaction volume. If less than 10^3 cells are used to prepare the lysate, the QRT PCR system can tolerate more lysate, up to 20% of the reaction volume.

2. Gene specific primers must be used and amplicon should be

4. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

5. Program the thermal cycler so that cDNA synthesis is followed immediately by QPCR amplification.

Steps	Temperature	Duration	Cycle (s)
cDNA Synthesis	42°C	30 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:

- Minimize RNA degradation by keeping cells in PBS on ice before starting the cell lysis procedure.
- Do not vortex Stop Solution.
- Lysis Solution and Stop Solution must be at room temperature before proceeding to the lysis procedure. As cells settle quickly, thoroughly resuspend cells before withdrawing cell solution samples.
- (Optional) If setting up multiple reactions, prepare Protease/Lysis Solution premix for the number of reactions required, and then mix the premix solution with 5 µl of 10-10^5 cells.
- (Optional) If setting up multiple reactions, prepare Protease Inhibitor / Stop Solution premix for the number of reactions required, and then mix the premix solution with the lysis reaction.
- As RNAs are poor templates for DNA polymerase, a Ct difference of 8-12 would be expected in QPCR between reactions containing RTase and those with no RTase.
- Lysates can be safely stored on ice for up to 1 hr after lysis. Alternatively, lysates can be stored at -80°C for a short period of time with a maximum of 1 freeze / thaw cycle. We highly recommend to use the lysates in downstream applications immediately after the 2 min termination.
- When handling 10^3 or more copy number of cells, ROX Referencing option could be turned off if applied by the QPCR instruments.

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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