Extraction-Free 1-Step RT-qPCR Kit

Cat. No.	Product Description	Size
CRTQ01-00	Extraction free 1-Step RT-qPCR Kit	100x 20 µL Reactions
CRTQ01-01	Extraction free 1-Step RT-qPCR Kit	500x 20 µL Reactions
CRTQ01-02	Extraction free 1-Step RT-qPCR Kit	5000x 20 µL Reactions

Intended Use

- The Master Mix is used for real-time qualitative and quantitative RT-PCR amplifications with SYBR Green dye directly from cell lysate. No RNA extraction is needed.
- The RT-qPCR master mix is a premixed, 2x concentrated solution that has all the components except for gene-specific primers and RNA template.

Kit Characterizations

- A unique lysis buffer is used to lyse the cultured cells
- The RT-qPCR master mix is specially formulated to overcome the endogenous PCR inhibition in extraction-free RNA samples.
- For the reverse transcription step, this kit uses a highly efficient Thermophilic Reverse Transcriptase (RTase, US patent pending), which is a thermophilic type A polymerase, with optimal working temperatures of 60-62°C.
- The RTase is easily heat-inactivated at ≥90°C for 1min.
- The RTase efficiently synthesizes a complementary DNA strand on RNA template from a gene-specific primer, *≤1 unit per 20µL of reaction.*
- The RTase is capable of reversely transcribing single digit copies of target RNA molecules consistently.
- The kit contains *Taq-Fast* DNA polymerase which extends more than 300 bases with relatively short cycling program. The preferable PCR product size is ≤150bp.
- The concentrations of the primers are variable depending on assay designs and thermocycling protocols (Table 1).
- The kit has three formulations: No ROX, Low ROX or High ROX

Kit Contents

Cat. No.	Lysis Buffer	RT-qPCR Mastermix
CRTQ01-00	10 ml	1 ml
CRTQ01-01	50 ml	5x 1 ml
CRTQ01-02	500 ml	50 ml

Transportation and storage

- The kit can be transported at below 4°C for up to 3 days.
- The kit should be kept stable in the dark at -20°C for ≤24 months with ≤10 times of freeze-thaw cycles. The kit can be stored at 4°C for a week.

Protocol for extraction-free RNA from cultured cells

1. Wash 10,000-20,000 adherent cells in a well or suspension cells in a medium with 1X PBS buffer.
2. Add 50-100ul Lysis Buffer to the well or to the collected cells, and leave for 5min.
3. Break down the cells through either sonicator, or vigorous vortex for 1 min.
4. Heat at 95ºC for 10min and then cool down to 4°C.
5. Up to 4µl of lysed RNA sample can be added to a 20ul final RT-PCR volume.

Note: the recovery rate of the extraction-free RNA is heavily affected by how completely the cells are broken down.

Setup Reaction and Thermocycling

1. Thaw 1-Step 2X RT-PCR Mastermix and other reaction components at room temperature, mix each component, centrifuge and then place on ice.
2. Set up reactions (Table 1).
3. Seal tubes with flat, optically transparent caps or seal plate with optically transparent film.
4. Mix and then briefly centrifuge the tubes or plate.
5. Program PCR instrument with indicated thermo-cycling protocol.

6. Load PCR tubes or plate and start to run.
7. Perform data analysis according to the PCR instrument instructions.

Table 1. Setting up a 20μL or 10μL reaction

Component	Volume per 20μL	Volume per 10μL	Final concentration
2x Mastermix	10μL	5μL	1X
Primers[a]	Variable	Variable	Each 150-900nM depending on primer design and thermocycling
RNA templates[c]	Variable	Variable	As low as single digit copies of target RNA to ≤1μg total RNA
H_2O	To 20μL	To 10μL	

Note: Each primer's T_m should be designed ≥60˚C, preferably between 62˚C to 65˚C, using primer3 software for high efficiency and specificity.

Table 2. Compatible instruments

qPCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye

Table 3. Standard Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	1min	1
III	95°C	10sec	35-40
	60°C, signal acquisition	60sec	
IV	60°C to 95°C	Various	1

Table 4. Fast Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	1min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	
IV	68°C to 95°C	Various	1

Footnotes of Tables 3 and 4

- The primer concentration used in Tables 3 and 4 is typically 0.15-0.2uM
- The Fast thermocycling protocol in Table 4 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 3.

Precautions

If you order a "**No ROX**" Mastermix but you have an Applied Biosystems or Thermo Fisher instrument, please **turn off ROX passive reference dye button** when setup assays.