TECHNICAL DATA SHEET

One-Step RT-qPCR

Catalog number/SKU: 24100, 24200, 24300

PRODUCT DESCRIPTION

Our One-Step RT-qPCR is designed to optimize quantitative assays and gene expression profiling through a unified protocol that consolidates reverse transcription and amplification into a single step. This kit offers consistent precision over extended cycles and in challenging matrices, achieving linear and highly sensitive quantification with RNA and DNA samples.

Components	Volume 100 rxn (SKU 24100)	Volume 200 rxn (SKU 24200)	Volume 1000 rxn (SKU 24300)	Storage T°
20X RT Enzyme	100 µL	200 µL	1 mL	-20°C
2X Mastermix	1 mL	2 x 1 mL	2 x 5 mL	
Nuclease-free water	1 mL	1.5 mL	2 x 5 mL	
20X ROX Low	100 µL	200 µL	1 mL	

PRODUCT APPLICATIONS

- Detection and quantification of RNA pathogens.
- Gene expression analysis and mRNA quantification.
- Quality control and food and environmental safety.

SHIPPING AND STORAGE

The One-Step RT-qPCR Kit is designed to be transported at 2–8 °C without loss of performance for up to 7 days. This reduces environmental impact by minimizing thermal packaging, eliminating dry ice, and simplifying logistics.

Storage recommendations:

- Store at –20 °C upon arrival.
- Avoid repeated freeze–thaw cycles
- Do not use damaged or expired components. If damage occurs during shipment, contact Kura Biotech technical support at sales@kurabiotech.com

STANDARD PCR RECIPE

Calculate the volume of reagents needed for each reaction. Typically, reactions are set up in 20 µL volumes.

Components	20 µL reaction	Final concentration
2X Mastermix	10 µL	1X
RT Enzyme 20X	1 µL	1X
20X ROX Low	1 µL	1X
Forward primer (10 µM)	0.8 µL	0.4 µM
Reverse primer (10 µM)	0.8 µL	0.4 µM
Probe (5 µM)	0.8 µL	0.2 µM
Template	variable*	-
Nuclease-free water	Up to 20 µL	-

***Template:** In samples of low purity or difficult matrices, diluting the sample is recommended to avoid the concentration of inhibitors.

PCR SETUP

1) Determine the appropriate total volume for the number of reactions. Add all components and the template at the end into a sterile PCR tube or plate, and mix by pipetting up and down. Centrifuge gently to collect all liquid at the bottom of the tube.
2) Seal the tubes with clear optical caps or the PCR plates with clear optical film, ensuring they are sealed correctly to prevent evaporation.
3) Gently centrifuge the tubes or plates to remove bubbles before placing them in the thermal cycler.

PCR CYCLING PROGRAM

Step	Temperature	Time	Cycles
RT Step	55°C	5 min	1
Initial Denaturation	95°C	2 min	1
Denaturation	95°C	15 sec	45
Annealing/ extension	60°C**	30 sec	

**Ensure that the annealing temperature corresponds to the Tm of your primers. Use a 2-step protocol if the Tm is >58 °C, combining annealing and extension in a single step as shown in the table; if the Tm is <58 °C, use a 3-step protocol, with an annealing step at the primers' Tm, followed by an extension at 72 °C.

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