QX700™ One-Step RT-ddPCR™ Kit for Probes

Catalog #	Description
12025316	**QX700 One-Step RT-ddPCR Kit for Probes**, 200 x 5 µL reactions
12025504	**QX700 One-Step RT-ddPCR Kit for Probes**, 500 x 5 µL reactions
12025531	**QX700 One-Step RT-ddPCR Kit for Probes**, 2,500 x 5 µL reactions

For research purposes only.

Description

The QX700 One-Step RT-ddPCR Kit for Probes enables precise 7-target multiplex RNA quantification using Droplet Digital™ PCR (ddPCR). The optimized enzyme blend includes Bio-Rad's proprietary Reliance Reverse Transcriptase, designed for improved specificity, sensitivity, and efficiency. The kit also includes an RNase inhibitor to ensure RNA protection throughout the workflow.

Kit Contents

QX700 One-Step RT-ddPCR Kit for Probes contains a supermix at 10x and enzyme mix at 10x (Table 1).

Table 1. Kit contents for QX700 One-Step RT-ddPCR Kit for Probes.

Kit Size	Supermix (10x)	Enzyme Mix (10x)
200 x 5 µL reactions	50 µL x 2	50 µL x 2
500 x 5 µL reactions	50 µL x 5	50 µL x 5
2,500 x 5 µL reactions	625 µL x 2	625 µL x 2

Storage and Stability

All components of the QX700 One-Step RT-ddPCR Kit for Probes are stable through the expiration date printed on the label when stored in a constant temperature freezer at –20°C. Repeated freezing and thawing of the supermix is not recommended. Once thawed, the 10x supermix can be stored at 4°C for up to 2 weeks. Keep the 10x enzyme mix at –20°C.

Please note: the 10x enzyme mix will remain in a liquid state at –20°C.

Quality Control

QX700 One-Step RT-ddPCR Kit for Probes is free of contaminating DNase and RNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

Recommendations for Optimal Results

- For optimal results, all components need to be vortexed as mentioned in Reaction Setup
- Follow general guidelines and recommendations for ddPCR
- Prepare the RNA sample before setting up the reverse transcription reaction mix and keep both on ice
- Suggested input quantities of total RNA are 250 fg–500 ng per 5 µL reaction

Reagents and Equipment

- QX700 E Droplet Digital PCR System* (catalog# 17011036), QX700 S Droplet Digital PCR System* (#17010638), or QX700 HT Droplet Digital PCR System* (#17010628)
- RDG16 Cartridges, Pack of 12 (#12025252)

* For Research Use Only. Not for use in diagnostic procedures.

Reaction Setup

1. Thaw the 10x supermix tube on a 37°C heating block for 1 minute to fully dissolve the solution. Keep the 10x supermix tube at room temperature. Vortex both tubes at maximum speed for 10 seconds to mix thoroughly, then briefly centrifuge to collect the contents at the bottom. Keep the 10x enzyme tube on ice before and after vortexing/centrifuging.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed according to the guidelines in Table 2. Assemble all required components except the sample, dispense equal aliquots into each reaction tube, and add the sample to each reaction tube as the final step.

Note: The reactions should be set up on ice before droplet generation to prevent a nonspecific reverse transcription reaction from occurring.

Table 2. Preparation of the reaction mix.

Kit Size	Volume per Reaction, µL	Final Concentration
Supermix (10x)	0.5	1x
Enzyme mix (10x)	0.5	1x
Target primers/probe*	Variable	900 nM/250 nM
RNase-/DNase-free water	Variable	—
Total RNA	Variable	up to 500 ng per reaction
Total volume	5	—

*Primers and probes must be target-specific hydrolysis probe assays from an authorized supplier, such as PrimePCR ddPCR Gene Expression Probe Assays.

4. Mix thoroughly by vortexing the reaction tubes at maximum speed for 10 seconds. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes.
5. Transfer 5 µL of the reaction mix from the reaction tube to the corresponding chamber in RDG16 cartridges.

Note: When using the QX700S and QX700E Droplet Digital PCR Systems, One-Step RT-ddPCR reactions must be set up in a single RDG48 plate and the plate must be loaded only as the first run. Up to two RDG48 plates can be loaded as the first run with the QX700HT Droplet Digital PCR System.

Thermal Cycling Conditions

Follow instructions based on the system in use:

- Proceed to thermal cycling (see Table 3)
- Refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493) and RDG16 Instructions for Use (10000171484) for sample and plate set up instructions. Use appropriate thermal cycling conditions as specified in Table 3.

Table 3. Thermal cycling conditions.

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Reverse transcription	50	15 min	1°C/sec	1
Enzyme activation	95	10 min		1
Denaturation	95	10 sec		45
Annealing/extension	55–65	1 min		45

Data Acquisition and Analysis

Table 4. Recommended exposure time.

Channel	Color	Times (msec)
1	Blue	120
2	Teal	273
3	Green	365
4	Yellow	337
5	Red	51
6	Infra-Red	470
7	Long-Shift	110

For the QX700 E Droplet Digital PCR System, QX700 S Droplet Digital PCR System, and QX700 HT Droplet Digital PCR System refer to the QX700 Droplet Digital PCR System Instrument Guide (10000171493).

Visit **bio-rad.com/QX700OneStepKit** for more information.

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