BIO-RAD

One-Step RT-ddPCR Advanced Kit for Probes

Catalog #	Description
1864021	**One-Step RT-ddPCR Advanced Kit for Probes**, 200 x 20 µl reactions
1864022	**One-Step RT-ddPCR Advanced Kit for Probes**, 500 x 20 µl reactions

For research purposes only.

Description

One-Step RT-ddPCR Advanced Kit for Probes delivers improved efficiency, specificity, and sensitivity for precise RNA target quantification by Droplet Digital™ PCR (ddPCR™). The optimized enzyme blend enables partitioning of RNA samples into droplets while keeping the enzymes inactive until the reverse transcription reaction is performed at 50°C. This enhances the specificity and efficiency by ensuring full enzyme activation for primer-mediated cDNA conversion. The supermix contains RNase inhibitor that protects the RNA throughout the entire workflow.

Kit Contents

One-Step RT-ddPCR Advanced Kit for Probes contains supermix, reverse transcriptase (RT), and 300 mM dithiothreitol (DTT) solution (Table 1).

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

Kit Size	Supermix	RT	DTT
200 x 20 µl reactions	500 µl x 2	200 µl x 2	1 ml x 2
500 x 20 µl reactions	500 µl x 5	200 µl x 5	1 ml x 5

Storage and Stability

All components of the One-Step RT-ddPCR Advanced Kit for Probes are stable for 12 months when stored in a constant temperature freezer at –20°C. Repeated freezing and thawing of the supermix is not recommended. DTT should be aliquoted to multiple tubes and stored at –20°C to minimize freezing and thawing.

Quality Control

One-Step RT-ddPCR Advanced 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 Droplet Digital PCR (refer to the Droplet Digital PCR Applications Guide, bulletin 6407)
- Prepare the RNA sample before setting up the reverse transcription reaction mix, and keep both of them on ice
- Suggested input quantities of total RNA are 100 fg–100 ng per reaction

Required Equipment

The QX200™ Droplet Digital PCR System (catalog #1864001), QX200 AutoDG™ Droplet Digital PCR System (#1864100), QX600™ Droplet Digital PCR System (#17007769), QX600 AutoDG Droplet Digital PCR System (#17008371), or QX ONE™ Droplet Digital PCR System (#12006536) is required.

Refer to the QX200 Droplet Reader and QX Manager Software Standard Edition User Guide and QX200 Droplet Generator Instruction Manual (10000107223 and 10031907, respectively), the Automated Droplet Generator Instruction Manual (10043138), or the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for ordering information about consumables, such as oils, cartridges, gaskets, plates, and seals.

Reaction Setup

1. Thaw all components on ice for 30 min. Mix thoroughly by vortexing each tube at **maximum speed for 30 sec** to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of each tube.
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.

Component	Volume per Reaction, µl	Final Concentration
Supermix	5	1x
Reverse transcriptase	2	20 U/µl
300 mM DTT	1	15 mM
Target primers/probe*	Variable	900 nM/250 nM
RNase-/DNase-free water	Variable	—
Total RNA	Variable	100 fg–100 ng per reaction
Total volume**	**20**	**—**

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

** For the Automated Droplet Generator, prepare 22 µl per reaction.

4. Mix thoroughly by vortexing the reaction tubes at **maximum speed for 10 sec**. Centrifuge briefly to ensure that all components are at the bottom of the reaction tubes. Allow reaction tubes to equilibrate to room temperature for no more than 10 minutes before droplet generation.
5. Transfer the reaction mix from the reaction tubes to the appropriate ddPCR Cartridge as follows:
   - For the QX600 or QX200 Droplet Digital PCR System, load 20 µl of each reaction mix into a sample well of a DG8 Cartridge. Follow subsequent instructions as specified in the QX200 Droplet Generator Instruction Manual (10031907)
   - For the QX600 or QX200 AutoDG Droplet Digital PCR System, follow instructions in the Automated Droplet Generator Instruction Manual (10043138)
   - For the QX ONE Droplet Digital PCR System, load 20 µl of each reaction mix into a sample well of a GCR96 Cartridge. Follow subsequent instructions as specified in the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512)

Note: When using the QX ONE ddPCR System, the One-Step RT-ddPCR reactions must be set up on a single GCR96 plate and the plate must be put in the first slot of the QX ONE Plate Inbox.

Thermal Cycling Conditions

Follow instructions based on the system in use:

- For the QX600 or QX200 Droplet Digital PCR System, after droplet generation with the QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate. Seal the plate using the PX1 PCR Plate Sealer (#1814000) at 180°C for 5 sec. Proceed to thermal cycling (see Table 3)
- For the QX600 or QX200 AutoDG Droplet Digital PCR System, remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate using the PX1 PCR Plate Sealer at 180°C for 5 sec. Proceed to thermal cycling (see Table 3)
- For the QX ONE Droplet Digital PCR System, thermal cycling is integrated into and sequentially performed by the system itself. Hence, no additional equipment or sample handling is required for this step. Refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) for plate setup instructions. Use appropriate thermal cycling conditions as specified in Table 3

Table 3. Thermal cycling conditions.*

Cycling Step		Temperature, °C	Time	Number of Cycles
Hold (QX ONE ddPCR System only)		25	3 min	1
Reverse transcription		42–50	60 min	1
Enzyme activation		95	10 min	1
Denaturation		95	30 sec	40
Annealing/extension		55–65	1 min**	40
Enzyme deactivation		98	10 min	1
Hold	QX600 or QX200 ddPCR System	4	30 min	1
	QX ONE ddPCR System	25	1 min	1

* For the PTC Tempo Deepwell Thermal Cycler or C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module, use a heated lid set to 105°C and set the sample volume to 40 µl.

** Check/adjust ramp rate settings to ~2°C/sec.

Data Acquisition and Analysis

Follow instructions based on the system in use:

- For the QX600 Droplet Digital PCR System and the QX600 AutoDG Droplet Digital PCR System, refer to the QX600 Droplet Reader and QX Manager Software Standard Edition User Guide (10000153877)
- For the QX200 Droplet Digital PCR System and the QX200 AutoDG Droplet Digital PCR System, refer to the QX200 Droplet Reader and QX Manager Software Standard Edition User Guide (10000107223)
- For the QX ONE Droplet Digital PCR System, refer to the QX ONE Droplet Digital PCR System and QX ONE Software User Guide (10000116512) and the QX ONE Software User Guide for Standard Edition (10000116655) or Regulatory Edition (10000116656)

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

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