

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.

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 at room temperature for about 3 min.
5. Once the reaction mixtures are ready, load 20 µl of each reaction mix into a sample well of a DG8™ Cartridge for QX200™/QX100™ Droplet Generator (catalog #1864008) followed by 70 µl of Droplet Generation Oil for Probes (catalog #1863005) into the oil wells, according to the QX100 or QX200 Droplet Generator Instruction Manual (#10026322 or 10031907, respectively). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138).

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

- QX100 or QX200 Droplet Generator (catalog #1863002 or 1864002, respectively) or Automated Droplet Generator (catalog #1864101)
- QX100 or QX200 Droplet Reader (catalog #1863003 or 1864003, respectively)
- C1000 Touch™ Thermal Cycler with 96-Deep Well Reaction Module (catalog #1851197)
- PX1™ PCR Plate Sealer (catalog #1814000)

Please refer to the QX100 or QX200 Instruction Manuals (#10026321 and 10026322 or 10031906 and 10031907, respectively) or the Automated Droplet Generator Instruction Manual (#10043138) for ordering information on consumables (oils, cartridges, gaskets, plates, and seals).

Thermal Cycling Conditions

1. After droplet generation with the QX100 or QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate, or remove the droplet plate containing ddPCR droplets from the Automated Droplet Generator. Seal the plate with the PX1 PCR Plate Sealer.
2. Proceed to thermal cycling (see protocol in Table 3) and subsequent reading of droplets in the QX100 or QX200 Droplet Reader.

Table 3. Cycling conditions for Bio-Rad's C1000 Touch Thermal Cycler.*

Cycling Step	Temperature, °C	Time	Number of Cycles
Reverse transcription	42–50	60 min	1
Enzyme activation	95	10 min	1
Denaturation	95	30 sec	40
Annealing/extension	55–65	1 min**	
Enzyme deactivation	98	10 min	1
Hold (optional)	4	∞	1

* Use a heated lid set to 105°C and set the sample volume to 40 µl.

** Check/adjust ramp rate settings to ~2–3°C/sec.

Data Acquisition and Analysis

1. After thermal cycling, place the sealed 96-well plate in the QX100 or QX200 Droplet Reader.
2. Open QuantaSoft™ Software to set up a new plate layout according to the experimental design. Refer to the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual (#10026321 or 10031906, respectively).
3. Under Setup, double click on a well in the plate layout to open the Well Editor dialog box.

4. Designate the sample name, experiment type, **ddPCR** as the supermix type, target name(s), and target type(s): **Ch1** for FAM and **Ch2** for HEX or VIC.
5. Select **Apply** to load the wells and, when finished, select **OK**.
6. Once the plate layout is complete, select **Run** to begin the droplet reading process. Select the appropriate dye set used and run options when prompted.
7. After data acquisition, select samples in the well selector under Analyze. Examine the automatic thresholding applied to the 1-D or 2-D amplitude data and, if necessary, set thresholds or clusters manually.
8. The concentration reported is copies/µl of RNA in the final 1x ddPCR reaction.

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

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