One-Step RT-ddPCR Kit for Probes

Catalog #	Supermix Volume	Kit Size
186-3021	2 ml (2 x 1 ml)	200 x 20 µl reactions
186-3022	5 ml (5 x 1 ml)	500 x 20 µl reactions

For research purposes only.

Storage and Stability
The components in the One-Step RT-ddPCR Kit for Probes are stable at –20°C through the expiration date printed on the labels. Repeated freezing and thawing of the supermix is not recommended.

Description
One-Step RT-ddPCR Supermix is a 2x concentrated, ready-to-use reaction cocktail containing all components — except manganese (supplied in separate tube), primers, probe(s), and template — required for probe-based Droplet Digital™ PCR (ddPCR™). The mixture delivers maximum target specificity and fluorescence amplitude with minimum droplet variability to ensure precise target quantification. Conventional cycling protocols are used for probe-based singleplex or duplex ddPCR.

The hot-start features of the enzyme blend in the One-Step RT-ddPCR Supermix enable partitioning of RNA samples into droplets while keeping the enzyme inactive at ambient conditions. Reverse transcription reaction is performed at 60°C, enhancing the specificity and efficiency by ensuring full enzyme activation for primer-mediated cDNA conversion. The thermostable enzymes allow for the RNA template to be reverse transcribed and subsequently amplified in the same reaction tube. The supermix also contains RNase inhibitor that protects the RNA throughout the entire workflow.

The One-Step RT-ddPCR Supermix is compatible with the use of uracil N-glycosylase (UNG) for PCR decontamination. UNG may be purchased from a licensed supplier.

Kit Contents
The One-Step RT-ddPCR Kit for Probes contains supermix and 25 mM manganese acetate solution (see Table 1).

Table 1. Kit sizes and volumes for the One-Step RT-ddPCR Kit for Probes.

Kit	Kit Size	Kit Contents and Volume		Function
		Supermix	25 mM Manganese Acetate Solution	
One-Step RT-ddPCR Kit for Probes	200 x 20 µl reactions	1.0 ml x 2	1.0 ml x 1	The 2x supermix is for use on Bio-Rad's ddPCR systems, for the hot-start, gene-specific, one-step RT-PCR amplification and detection of RNA targets using commercially available hydrolysis probe–based assays.
	500 x 20 µl reactions	1.0 ml x 5	1.0 ml x 2	

Quality Control
The One-Step RT-ddPCR Supermix is free of contaminating DNase and RNase. Stringent specifications are maintained to ensure lot-to-lot consistency.

Recommendations for Optimal Results
- Follow general guidelines and recommendations for ddPCR
- Suggested input quantities of template are: 5 ng to 50 fg per reaction

Important: For optimal results, design assays with a T_m of 60°C (or higher for GC-rich targets). T_m should be selected based on thermodynamics guidelines established by SantaLucia (SantaLucia 1998). Amplicon lengths should also be 60–150 bp for optimal RT-ddPCR efficiency.

Required Equipment
- QX100™ or QX200™ Droplet Generator (catalog #186-3002 or 186-4002)
- QX100 or QX200 Droplet Reader (catalog #186-3003 or 186-4003)
- C1000™ Touch Thermal Cycler (catalog #185-1196)
- PX1™ PCR Plate Sealer (catalog #181-4000)

Please refer to the QX100 or QX200 manuals for ordering information on consumables (oils, cartridges, gaskets, plates and seals, etc.).

Reaction Setup

1. Thaw all components at room temperature. Mix thoroughly by inverting the tubes several times to ensure homogeneity, as a concentration gradient may form during –20°C storage. Centrifuge to collect contents at the bottom of the tube.
2. Prepare RNA templates at the desired concentration before setting up the RT-ddPCR reaction mix, and keep on ice.
3. Prepare the RT-ddPCR reaction mix for the appropriate number of reactions needed according to the guidelines in Table 2. Assemble all required components except the RNA template, dispense equal aliquots into each reaction tube, and add the template to each reaction tube as the final step.

Table 2. Preparation of the RT-ddPCR reaction mix.

Component	Volume per Reaction, µl	Final Concentration
2x one-step RT-ddPCR supermix	10	1x
25 mM manganese acetate solution	0.8	1 mM
Forward primer	Variable	500–900 nM*
Reverse primer	Variable	500–900 nM*
Fluorogenic probe	Variable	250 nM
RNase/DNase-free water	Variable	--
RNA template	Variable	5 ng to 50 fg per reaction
Total volume	**20 µl**	**--**

*For duplex assays with large copy number differences, the primer concentration of the lower copy target can be increased to up to 900 nM to achieve optimal results.

4. Mix thoroughly by briefly vortexing the tube or by pipetting the mix up and down 5x, and centrifuging briefly to ensure that all components are at the bottom of the reaction tube.
5. Once the reaction mixtures are ready, allow the reaction tubes to equilibrate at room temperature for about 3 minutes before loading 20 µl of each reaction mix into a sample well of a DG8™ Cartridge (catalog #186-4008), according to the Droplet Generator manual.

Cycling Conditions for RT-ddPCR

After droplet generation with the QX100 or QX200 Droplet Generator, carefully transfer droplets into a clean 96-well plate for sealing with the PX1 PCR Plate Sealer, thermal cycling (see protocol in Table 3), and subsequent reading of droplets in the QX100 or QX200 Droplet Reader.

Table 3. Cycling protocol for Bio-Rad® C1000 Touch Thermal Cycler.*

Cycling Step	Temperature, °C	Time	Ramp Rate	# Cycles
Reverse transcription	60	30 min	Approximately 2.0°C/sec	1
Enzyme activation	95	5 min		1
Denaturation	94	30 sec		40
Annealing/Extension	60	1 min		
Enzyme heat kill	98	10 min		1
Hold (optional)	4	Infinite		1

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

To learn more about Bio-Rad's complete solution for amplification, visit our website: **www.bio-rad.com/amplification**

Reference:
SantaLucia J (1998). A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. Proc Natl Acad Sci USA 95, 1460-1465.

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