

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, $^{\circ}\text{C}$	Time	Ramp Rate	# Cycles
Reverse transcription	60	30 min	Approximately 2.0 $^{\circ}\text{C}/\text{sec}$	1
Enzyme activation	95	5 min		1
Denaturation	94	30 sec		40
Annealing/Extension	60	1 min		1
Enzyme heat kill	98	10 min		1
Hold (optional)	4	Infinite		1

*Use a heated lid set to 105 $^{\circ}\text{C}$ 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.

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product may be covered by one or more of the following U.S. patents: 5,789,224; 5,618,711; 6,127,155; 5,677,152 (claims 1 to 23 only); 5,773,258 (claims 1 and 6 only); 5,804,375; 5,538,848; 5,723,591; 5,876,930; 6,030,787; 6,258,569; and claims outside the United States corresponding to expired U.S. Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The QX100/QX200 Droplet Digital PCR system and/or its use is covered by claims of U.S. patents, and/or pending U.S. and non-U.S. patent applications owned by or under license to Bio-Rad Laboratories, Inc. Purchase of the product includes a limited, non-transferable right under such intellectual property for use of the product for internal research purposes only. No rights are granted for diagnostic uses. No rights are granted for use of the product for commercial applications of any kind, including but not limited to manufacturing, quality control, or commercial services, such as contract services or fee for services. Information concerning a license for such uses can be obtained from Bio-Rad Laboratories. It is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required.

Thermal cyclers and real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367. Purchase of this instrument conveys a limited non-transferable immunity from suit for the purchaser's own internal research and development and for use in human in vitro diagnostics and all other applied fields under U.S. Patent Number 5,475,610 (Claims 1, 44, 158, 160–163, and 167 only), or corresponding claims in its non-U.S. counterpart, owned by Applera Corporation. No right is conveyed expressly, by implication, or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA. FAM and VIC are trademarks of Applera Corporation.