Manual

RapiDxFire Thermostable Reverse Transcriptase

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GENOMIC ANALYSIS BY LGC

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1. Introduction

RapiDxFire™ Thermostable Reverse Transcriptase is a proprietary enzyme that performs fast and efficient first strand cDNA synthesis at higher temperatures than standard AMV- and MMLV-based reverse transcriptases (RT). Originally identified from viral DNA isolated from hot springs, this enzyme derivative lacks RNAse H activity and has strong and increasing reverse transcription activity from 50 ºC to 80 ºC, maintaining approximately 60% of the activity after 10 minutes at 90 ºC. RapiDxFire Thermostable RT synthesises cDNAs up to 1000 nt from diverse RNA templates in 5 minutes using gene-specific primers of appropriate melting temperature (Tm). As few as 100 copies of RNA can be detected in subsequent PCR after conversion to cDNA. The enzyme formulation does not contain glycerol and is compatible with lyophilisation. RapiDxFire Thermostable Reverse Transcriptase is produced by over-expression in *E. coli* and purified in a quality standard ISO 13485-certified manufacturing facility.

Applications: Rapid and sensitive first-strand cDNA synthesis (<1 Kb).

2. Kit contents

Product	Kit size	Cat no.	Reagent description	Part no.	Volume
RapiDxFire Thermostable Reverse Transcriptase	50 Rxn @ 25 µL each	30250-1	RapiDxFire Thermostable Reverse Transcriptase, 3 RT Units/µL	F735110-1	50 µL
			10X Thermostable RT Buffer	F735111-1	250 µL
RapiDxFire Thermostable Reverse Transcriptase	250 Rxn @ 25 µL each	30250-2	RapiDxFire Thermostable Reverse Transcriptase, 3 RT Units/µL	F735110-2	250 µL
			10X Thermostable RT Buffer	F735111-2	1.25 mL

All kit components are available in bulk volumes and custom packaging.

3. Product specifications

Storage: Store at -20 °C.

Storage Buffer: RapiDxFire Thermostable RT is supplied in a glycerol-free, Triton X-100-free buffer.

Unit Definition: One RT unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 55 °C (using poly(rA) • oligo(dT) as template primer and 1x kit -supplied buffer formulation).

Quality Control: RapiDxFire Thermostable RT is functionally tested with gene-specific primer and bacteriophage MS2 RNA. In this reaction the enzyme converts 1000 copies of MS2 RNA into 520 bp cDNA in 5 minutes at 60 °C.

Contaminating Activity Assays: RapiDxFire Thermostable RT enzyme and 10x Thermostable RT buffer are free of detectable contaminating DNA exonuclease, DNA endonuclease, and RNases.

4. Customer provided reagents

1. dNTP mix
2. Gene-specific primer of appropriate Tm
3. Purified template RNA or Total RNA
4. Nuclease-free water
5. NxGen RNase Inhibitor, Cat #30281 (Optional)

5. General guidelines

1. The enzyme performs best when generating cDNA lengths of ≤500 nt. cDNA lengths of up to 1000 nt can be achieved but may require longer reaction times or higher reaction temperatures.
2. Use gene-specific primers. Random primers and Oligo (dT) primers will not anneal efficiently at higher reaction temperatures.
3. Reaction conditions will vary for different primers and targets. A 60 °C reaction temperature will work well for most RNA templates and serves as a good starting point for both primer design (length) and enzyme performance. Make sure that your gene-specific primer has a Tm appropriate to your chosen reaction temperature.
4. Use good laboratory practices when handling RNA. Wear gloves and use nuclease-free tips and reagents.
5. The use of an RNase inhibitor is optional. If needed, we strongly recommend using NxGen® RNase Inhibitor (Cat # 30281) at a final concentration of 0.5 U/µL. Our data suggests that other RNase inhibitors (including RiboGuard™ RNase inhibitor) may interfere with the reaction performance.
6. Use a heated thermal cycler lid when incubating thermostable RT reactions to prevent evaporation and condensation of small reaction volumes.
7. Enzyme and buffer formulations are compatible with lyophilisation, but excipient and protocol optimisation will be required based on final application.

6. Reaction set-up

The reaction set-up detailed in Table 1 is intended for guidance only. Conditions will vary for different primers and targets. The reaction volume is scalable to customer's needs.

Component	Initial concentration	Final concentration	Volume (25 µL reaction)
10x Thermostable RT Buffer	10x	1x	2.5 µL
dNTPs	25 mM	0.5 mM	0.5 µL
Gene-specific primer	10 µM	0.4 µM	1.0 µM
RapiDxFire Thermostable Reverse Transcriptase	3 Units/µL	0.12 Units/ µL	1.0 µL
Template RNA (Low EDTA buffer)	varies	as required	as required
Nuclease-free water	NA	to 25 µL	to 25 µL
Total (µL)			25 µL

Table 1: Example first strand cDNA synthesis reaction set-up using RapiDxFire Thermostable Reverse Transcriptase

7. Protocol

1. Completely thaw all reaction components and place on ice for setup. Before use, vortex components and briefly spin the tubes in a microcentrifuge to ensure that the material is collected at the bottom of the tubes.
2. Prepare master mixes in sterile, nuclease-free microcentrifuge tubes on ice. For each sample or condition, prepare one master mix by multiplying each component volume by the total number of desired reactions (plus extra). Vortex the master mix and aliquot one reaction volume into each reaction tube.
3. Briefly spin the reaction tubes in a microcentrifuge to ensure that the material is collected at the bottom of the tubes.
4. Place the reaction tubes in a thermal cycler at the desired temperature and incubate for 5 minutes.
   Note: In most cases < 5 min will yield enough cDNA for downstream applications. Longer cDNA synthesis may benefit from longer reaction times, up to 20 minutes.
5. To remove the activity of the enzyme for downstream applications, heat-kill at 95 °C for 5 minutes (optional).
6. The cDNA can be used immediately, without purification, for end-point or real-time PCR (qPCR), converted to double-stranded cDNA, or stored at -20 °C for future use.

8. Ordering information

Cat No.	Product name	Description
30250-1	RapiDxFire Thermostable Reverse Transcriptase, 50 Reactions	3 RT units/µL
30250-2	RapiDxFire Thermostable Reverse Transcriptase, 250 Reactions	3 RT units/µL
30281-1	NxGen® RNase Inhibitor (optional)	10,000 units
30281-2	NxGen® RNase Inhibitor (optional)	50,000 units

For any queries about this guide please contact:
All locations except USA: email tech.support@lgcgroup.com or call +44 (0)1992 476 486
USA only: email us-support@lgcgroup.com or call +1 978 338 5317

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