Manual

RapiDxFire Hot Start Taq DNA Polymerase

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GENOMIC ANALYSIS BY LGC

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1. Introduction

RapiDxFire™ Hot Start Taq DNA Polymerase contains recombinant Taq DNA Polymerase and select blockers that inhibit polymerase activity at temperatures below 60 °C. The polymerase activity is restored after an initial 94 °C denaturation step. Inhibition of polymerase activity during room temperature prevents the amplification of non-specific products (e.g. primer dimers) and improves the amplification of target DNA. RapiDxFire Hot Start Taq DNA Polymerase possesses 5′→3′ DNA polymerase activity and has non-template dependent, terminal transferase activity that adds a single deoxyadenosine to the 3′ ends of PCR products. The polymerase has dsDNA specific 5′→3′ exonuclease activity but does not have 3′→5′ exonuclease activity. This product is formulated in either a standard glycerol-containing storage buffer or a glycerol-free (GF) storage buffer (compatible with lyophilisation.)

Applications: PCR, RT-qPCR, qPCR

2. Kit contents

Product	Kit size	Cat no.	Reagent description	Part no.	Volume
RapiDxFire Hot Start Taq	1000 Units @ 5 U/µL	30042-1	RapiDxFire Hot Start Taq, 1000 Units at 5 U/µL	F835402-1	200 µL
			10X Hot Start Taq Buffer	F835248-1	2 x 2.0 mL
			25 mM $MgCl_2$	F95374-1	2 x 1.5 mL
RapiDxFire Hot Start Taq GF	1000 Units @ 5 U/µL	30043-1	RapiDxFire Hot Start Taq GF, 1000 Units at 5 U/µL	F835401-1	200 µL
			10X Hot Start Taq Buffer	F835248-1	2 x 2.0 mL
			25 mM $MgCl_2$	F95374-1	2 x 1.5 mL
RapiDxFire Hot Start Taq GF	500 Units @ 50 U/µL	30044-1	RapiDxFire Hot Start Taq GF, 500 Units at 50 U/µL	F835400-1	10 µL
			10X Hot Start Taq Buffer	F835248-1	2.0 mL
			25 mM $MgCl_2$	F95374-1	1.5 mL

3. Product specifications

Concentration: 5 U/µL (Cat no. 30042-1, 30043-1) or 50 U/µL (Cat. no. 30044-1).
Storage: Store at -20 °C.
Storage buffer: RapiDxFire Hot Start Taq is supplied in either 50% glycerol, Triton™ X-100-free buffer (Cat no. 30042-1) or glycerol-free, Triton™ X-100-free buffer (Cat no. 30043-1, 30044-1).

Unit definition: One unit catalyses the incorporation of 10 nmol of dNTP into acid-soluble material in 30 minutes at 70 °C in 50 mM Tris-HCl (pH 9.0), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [^{32}P] dCTP), 10 µg of activated calf thymus DNA, and 0.1 mg/ml BSA.

Quality control: RapiDxFire Hot Start Taq DNA Polymerase is functionally tested with gene-specific primers and *E.coli* genomic DNA.

Contaminating activity assays: RapiDxFire Hot Start Taq DNA Polymerase and 10X Hot Start Taq buffer are free of detectable contaminating DNA exonuclease, DNA endonuclease, and RNases.

Purity: Recombinant Taq DNA Polymerase is >95% pure as determined by SDS PAGE.

4. Customer provided reagents

1. dNTP mix
2. Primers
3. Template DNA
4. Nuclease-free water

5. General PCR guidelines

1. The following reaction set-up and cycling protocol should be considered a general guideline. The concentration of primers, $MgCl_2$, dNTPs, amount of RapiDxFire™ Hot Start Taq DNA Polymerase, and cycling conditions may need to be optimised on a per target basis.
2. PCR primers should be 18-22 bases in length to provide target specificity and a sufficient melting temperature. Both primers should have similar melting temperatures so that they anneal to the target at the same temperature. Primer melting temperature is generally 52-58 °C, with GC content between 40-60%. The 3´ ends should not be complementary to avoid possible primer-dimer formation. Avoid three G or C nucleotides in a row near the 3´ end of the primer, which may result in nonspecific primer annealing. Both primers should have similar melting temperatures; therefore, the primers should anneal at a similar temperature.
3. PCR template DNA can be from any source, such as genomic DNA, complementary DNA, or plasmid DNA. Optimal template amounts vary based on composition, complexity, and purity.
4. To minimise environmental contamination, prepare reactions (minus template) in a DNA-free location with dedicated pipettors and barrier tips. As the final step add template to the reactions in a separate location.

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 (50 µL reaction)
10X Hot Start Taq Buffer	10x	1x	5.0 µL
dNTPs	25 mM	0.2 mM	0.4 µL
$MgCl_2$ solution	25 mM	1.0-4.0 mM	X µL
Primer mix (containing forward and reverse primer in equal parts)	10 µM	0.1-1 µM	Y µL
RapiDxFire Hot Start Taq DNA Polymerase	5 Units/µL*	1.25 U	0.25 µL
Template DNA	varies	as required	as required
Nuclease-free water	NA	to 50 µL	to 50 µL
Total (µL)			50 µL

Table 1: Example PCR reaction set-up using RapiDxFire Hot Start Taq DNA Polymerase (5 U/µL)

*For 30044-1, dilute from 50 Units/µL to 5 U/µL for this setup guide.

7. Protocol

1. Completely thaw all reaction components. 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 at ambient temperature. 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 in a thermal cycler and start the desired PCR cycling conditions.

Step	Temperature	Time
Initial denaturation and Taq activation	94 °C	15 sec to 2 min
PCR cycling (25-40 cycles) Denaturation Annealing Extension	 94 °C 55 °C-65 °C 68 °C-72 °C	 10 to 30 sec 30 sec 1 min per kb of product length
Hold	4 °C	hold

8. Thermal cycling protocol

Full activation of RapiDxFire Hot Start Taq DNA Polymerase occurs within 15 seconds at 94 °C; however, complete denaturation of double stranded template is required. Due to this the initial denaturation time may require optimisation depending on the properties of a given target.

Annealing temperature conditions should be optimised starting with a temperature approximately 5 °C below the calculated melting temperature of the primers and titrating the temperature in 1 degree increments.

Allow the extension reaction to run for approximately 1 minute per kilobase of product, with a recommended final extension of 5 minutes.

9. Ordering information

Cat no.	Product name
30042-1	RapiDxFire Hot Start Taq, 1000 Units at 5 U/µL
30043-1	RapiDxFire Hot Start Taq GF, 1000 Units at 5 U/µL
30044-1	RapiDxFire Hot Start Taq GF, 500 Units at 50 U/µL

For any queries about this guide please email:
techsupport@lgcgroup.com

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