Manual

RapiDxFire qPCR 5X Master Mix with dUTP/UNG (and ROX)

For Laboratory Use.

BIOSEARCH™ TECHNOLOGIES

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1. Intended use

RapiDxFire™ qPCR 5X Master Mix contains low-glycerol, Triton-free buffer, dNTPs, thermostable hot start Taq DNA Polymerase, and other components specifically formulated for fast, reproducible multiplexing qPCR. In addition, RapiDxFire qPCR Master Mix with UNG and ROX contains uracil-DNA glycosylase to aid in contamination control caused by carryover from amplified products and ROX, a passive reference dye that enables fluorescent normalisation for qPCR.

RapiDxFire™ qPCR 5X Master Mix is intended for use as a general purpose reagent in molecular diagnostic assays that are based on qPCR target detection technologies by clinical laboratory professionals.

This product is classified as a general purpose in vitro diagnostic device reagent in the United States as defined in 21CFR 864.4010.

2. Product description

Key features of RapiDxFire qPCR 5X Master Mix with UNG include:

- 5X formulation offering flexible reaction setups and protocols
- dUTP and uracil-DNA glycosylase included
- Optional ROX passive reference dye
- Sensitive detection down to ~10 genomic DNA copies
- Wide dynamic range for multiplexing
- Low glycerol (0.01%), Triton-free, high concentration, and bulk formulation for adaptable test development and lyophilisation options
- Manufactured in an ISO 13485-certified facility demonstrating batch to batch reproducibility.

3. Warnings and Limitations

For Laboratory Use. For Professional Use.

The FLU label statement identifies an in vitro diagnostic medical device in the United States. Outside the United States, FLU is the equivalent of Research Use Only labeling.

This product must be qualified and validated by clinical laboratory end-users for suitability in the detection of any specific target using the procedures in this Manual as guidance.

4. Product designations

Product	Size	Catalog number	Reagent description	Part number
RapiDxFire qPCR 5X Master Mix with UNG	1 mL	30051-1	RapiDxFire qPCR 5X Master Mix with UNG, 1 mL	F837109-1
	10 mL	30051-2	RapiDxFire qPCR 5X Master Mix with UNG, 10 mL	F837109-2
RapiDxFire qPCR 5X Master Mix with UNG and ROX	1 mL	30053-1	RapiDxFire qPCR 5X Master Mix with UNG and ROX, 1 mL	F837120-1
	10 mL	30053-2	RapiDxFire qPCR 5X Master Mix with UNG and ROX, 10 mL	F837120-2

5. Product storage and specifications

Storage: store at -20 °C in a freezer without a defrost cycle.

Freeze-thaw cycles: *avoid repeated freeze-thaw cycles (<10 cycles).*

6. Materials supplied by the user:

- DNA target sequence-specific primers and probes of appropriate Tm
- Template DNA
- Molecular-grade, nuclease-free water
- PCR microtiter plates/tubes
- Optical plate seal
- qPCR instrument (with filters appropriate for selected dyes)

7. Before you start:

Reaction preparation and handling

- For quantification and/or concentration/copy number determination, it is recommended to follow the MIQE guidelines for qPCR.
- Reaction conditions will vary for different primers/probes and targets. A 55 °C to 62 °C annealing temperature will work for most DNA targets. Make sure that the target-specific oligonucleotides have a Tm appropriate for your chosen reaction temperature.
- Always use good laboratory practice. Wear gloves and use nuclease-free tips and reagents.

8. Protocol for qPCR

The protocol provides volumes for a single standard 20 µL reaction.

For alternate reaction sizes, scale component volumes proportionally.

To prepare a master mix, scale the component volumes to the desired reaction number. Include an extra 10% volume to account for pipetting.

1. Thaw all reaction components completely, then vortex briefly and centrifuge to collect contents at the bottom of the tubes. Keep tubes on ice or cooling block at all time when not in use.
2. Combine the components in a sterile, nuclease free tube on ice in the order listed in the table below.

Component	Volume	Final concentration
Nuclease-free water[1]	to 20 µL	N/A
RapiDxFire qPCR 5X Master Mix with dUTP/UNG and (ROX)	4 µL	1X
Forward primer[2]	0.6 µL	0.3 µM
Reverse primer[2]	0.6 µL	0.3 µM
Probe[2]	0.4 µL	0.2 µM
Template DNA	X µL	N/A

[1] The amount of nuclease-free water should be adjusted based on the volume of DNA Template.
[2] Alternatively, a 20X assay mix containing the necessary target specific oligos can be used at a 1X final concentration.

3. Perform PCR using standard PCR cycling protocols, with an additional UNG pre-thermocycling step.

   The following thermal cycling protocol is for guidance only, for assays designed under standard conditions, using good-quality DNA. When working with non-standard assay design or with more complex target sequences, further optimisation may be required including an additional annealing step or a different extension temperature.

Step	Temperature	Time	Number of cycles
1. UNG	50 °C	2 minutes	1 cycle
2. Hot start Taq activation	95 °C	2 minutes	1 cycle
3. Template denaturation	95 °C	3 seconds	45 cycles
4. Primer annealing and extension	60 °C	30 seconds	

9. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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