RapiDxFire qPCR 5X Master Mix GF quick guide

1. Thaw components at room temperature and mix well by vortex prior to use.
2. Prepare stock (100 µM) oligonucleotides by multiplying the nmol amount (e.g. 14.2 nM) by 10 (14.2 x 10 = 142). This is the volume of diluent, in µL, (142 µL) to be added to the tube.
3. Prepare working assay mixes as described in Table 1:

Component	40x assay mix (for final reaction volumes ≥5 µL)		80x assay mix (for final reaction volumes <5 µL)	
	Volume	Working concentration	Volume	Working concentration
100 µM primer (each)	20 µL	20 µM	40 µL	40 µM
100 µM probe (each)	8 µL	8 µM	16 µL	16 µM
Diluent	To 100 µL	-	To 100 µL	-
Total volume	100 µL	-	100 µL	-

Table 1. Preparation of 40x and 80x working assay mixes to allow for assay set-up with final oligonucleotide concentrations of 500 nM primer and 200 nM probe.

4. Prepare reaction mixes, for either singleplex (Table 2) or multiplex (Table 3) reactions.

Component	1.6 µL	5 µL	10 µL	25 µL	Final concentration
RapiDxFire qPCR 5X Master Mix GF	0.32 µL	1 µL	2 µL	5 µL	1X
Assay mix (40x or 80x)	0.02 µL (using 80x assay mix)	0.125 µL (using 40x assay mix)	0.25 µL (using 40x assay mix)	0.625 µL (using 40x assay mix)	500 nM primer, 200 nM probe
Template DNA	No more than 1.26 µL	No more than 3.375 µL	No more than 7.75 µL	No more than 19.375 µL	As required
Water*	-	To 5 µL	To 10 µL	To 25 µL	-

Table 2. Example of a singleplex reaction set-up. *Volume of water to be adjusted to account for any addition of passive reference dye

Component	1.6 µL	5 µL	10 µL	25 µL	Final concentration
RapiDxFire qPCR 5X Master Mix GF	0.32 µL	1 µL	2 µL	5 µL	1X
Assay mix (40x or 80x)	0.02 µL (using 80x assay mix per assay)	0.125 µL (using 40x assay mix per assay)	0.25 µL (using 40x assay mix per assay)	0.625 µL (using 40x assay mix per assay)	500 nM primer per assay, 200 nM probe per assay
Template DNA	No more than 1.26 µL	No more than 3.375 µL	No more than 7.75 µL	No more than 19.375 µL	As required
Water*	-	To 5 µL	To 10 µL	To 25 µL	-

Table 3. Example of a multiplex (duplex) reaction set-up. *Volume of water to be adjusted to account for any addition of passive reference dye

5. Place the reaction tubes/plates in a qPCR instrument and run the desired qPCR protocol (Table 4). Ensure instrument is set to read at the appropriate channels for the selected probes.

Step	Temperature	Time	Number of cycles
1	95 °C	2 minutes	1
2*	95 °C	15 seconds	40
	60 °C	1 minute	
	60 °C	Read	

Table 4. Guide for thermal cycling protocol for qPCR. *Step 2 can be modified to account for the specific Tm of the primers/probes in the specific assay.

For any queries about this quick guide, please contact techsupport@lgcgroup.com

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