RapiDxFire 1-step RT-qPCR System quick protocol

*For specific guidance on SARS-CoV-2 testing, please refer to the **RapiDxFire™ 1-step RT-qPCR System for SARS-CoV-2 detection quick protocol**.*

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 or multiplex reactions (Table 2).

Component	1.6 µL*[3]	5 µL	10 µL	20 µL	25 µL	Final concentration
RapiDxFire qPCR 5X Master Mix	0.32 µL	1 µL	2 µL	4 µL	5 µL	1X
EpiScript™ RNase H- Reverse Transcriptase, 200 U/µL	0.04 µL	0.125 µL	0.25 µL	0.5 µL	0.625 µL	5 U/µL
Assay mix*[1] (40x or 80x)	0.02 µL (80x assay mix)	0.125 µL (40x assay mix)	0.25 µL (40x assay mix)	0.5 µL (40x assay mix)	0.625 µL (40x assay mix)	500 nM primer, 200 nM probe
Template RNA	No more than 1.26 µL	No more than 3.375 µL	No more than 7.75 µL	No more than 16.5 µL	No more than 19.375 µL	As required
SuperROX (optional)	0.01 µL	0.03 µL	0.07 µL	0.13 µL	0.17 µL	100 nM
Water*[2]	-	to 5 µL	to 10 µL	to 20 µL	to 25 µL	-

Table 2. Example of a singleplex or multiplex reaction set-up.
*[1]40x or 80x assay mix volumes to be added for each assay per target, per reaction
*[2]Volume to be adjusted to account for any addition of passive reference dye.
*[3]If working with Array Tape™ platforms from LGC, Biosearch Technologies™ (e.g. IntelliQube™, Nexar™), generation of a 2X mix is recommended for pipetting accuracy. Please contact techsupport@lgcgroup.com for further guidance.

5. Place the reaction tubes/plates in a qPCR instrument and run the desired qPCR protocol (Table 3). Ensure instrument is set to read at the appropriate channels for the selected probes.

Step	Temperature	Time	Number of cycles
1	42 °C	15 minutes	1
2	95 °C	2 minutes	1
3*	95 °C	3 seconds	45
	55 °C-60 °C	30 seconds	

Table 3. Guide for thermal cycling protocol, for RT-qPCR.
*Step 3 can be modified to account for the specific Tm of the primers/probes in the specific assay

For any queries about this quick protocol, please visit our RapiDxFire 1-step RT-qPCR System webpage or contact techsupport@lgcgroup.com

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