RapiDxFire Lyo-Flex 1-Step RT-qPCR 5X Master Mix quick protocol

1. Thaw components at room temperature, and place on ice during set-up. Before use, pipette the RapiDxFire™ Lyo-Flex 1-Step RT-qPCR 5X Master Mix. Vortex all other components.
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 10X working primer/probe assay mix at the appropriate concentrations for each assay (example shown in Table 1).

Component	1X assay concentration	10X working concentration	10X working concentration volume
100 µM primer (each)	0.5 µM	5 µM	5 µL
100 µM probe (each)	0.2 µM	2 µM	2 µL
Diluent	-	-	To 100 µL
Total volume	**-**	**-**	**100 µL**

Table 1. Example for preparation of 10X working assay mix to allow for assay set-up with final oligonucleotide concentrations of 500 nM primer and 200 nM probe.

4. For testing with **wet** RapiDxFire Lyo-Flex 1-Step RT-qPCR 5X Master Mix, prepare reaction mix as described in Table 2 (20 µL total volume). For multiple targets, add the appropriate volume of 10X assay mix per assay. Keep the reaction mix on ice until use.

Component	1X reaction	Final concentration
RapiDxFire Lyo-Flex 1-Step RT-qPCR 5X Master Mix	4 µL	1X
Assay Mix (10X)	2 µL	1X primer/1X probe
Template RNA*	As required	As required
Water**	To 20 µL	-

Table 2. Example of a reaction set-up concentrations and volumes for RT-qPCR using wet RapiDxFire Lyo-Flex 1-Step RT-qPCR 5X Master Mix. *Template RNA can be added to bring the reaction to the desired total volume but may vary for different sample matrices i.e. those with inhibitory effects. **Volume of water to be adjusted to account for any addition of passive reference dye.

5. For testing with **lyophilised** RapiDxFire Lyo-Flex 1-Step RT-qPCR 5X Master Mix pellets, prepare reaction mix as described in Table 3 to rehydrate the lyophilised pellets (20 µL total volume). For multiple targets, add the appropriate volume of 10X assay mix per assay. Keep the reaction mix on ice until use.

Component	1X reaction	Final concentration
Assay Mix (10X)	2 µL	1X primer/1X probe
Template RNA*	As required	As required
Water**	To 20 µL	-

Table 3. Example of a reaction set-up concentrations and volumes for RT-qPCR using lyophilised RapiDxFire Lyo-Flex 1-Step RT-qPCR 5X Master Mix pellets. *Template RNA can be added to bring the reaction to the desired total volume but may vary for different sample matrices i.e. those with inhibitory effects. **Volume of water to be adjusted to account for any addition of passive reference dye.

6. 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	Description	Temperature	Time	Number of cycles
1	RT activation	85 °C	3 minute	1
2*	Reverse transcription	60 °C	5-15 minutes	1
3	hs*Taq* activation	95 °C	2 minutes	1
4	Denaturation	95 °C	10 seconds	45
	Annealing/extension	60 °C	30 seconds	

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

For additional information, please refer to the manual available on the Biosearch Technologies website.

For any queries regarding this quick protocol, please contact techsupport@lgcgroup.com

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