

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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