BHQ Probe Master Mix quick guide

1. Thaw all components on ice, and set up all reactions on ice.
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)	36 µL	36 µM	36 µL	36 µM
100 µM probe (each)	8 µL	8 µM	8 µL	8 µM
Diluent	To 100 µL	-	To 50 µL	-
Total volume	100 µL	-	50 µL	-

Table 1. Preparation of 40x and 80x working assay mixes to allow for assay set-up with final oligonucleotide concentrations of 900 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
2X BHQ Probe Master Mix	0.8 µL	2.5 µL	5 µL	12.5 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)	900 nM primer, 200 nM probe
Template DNA	0.8 µL	No more than 2.23 µL	No more than 4.45 µL	No more than 11.88 µL	As required
Water	-	To 5 µL	To 10 µL	To 25 µL	-

Table 2. Example of a singleplex reaction set-up.

Component	1.6 µL	5 µL	10 µL	25 µL	Final concentration
2X BHQ Probe Master Mix	0.8 µL	2.5 µL	5 µL	12.5 µL	1X
Assay mix (40x or 80x)	0.01 µL (using 80x assay mix per assay)	0.063 µL (using 40x assay mix per assay)	0.125 µL (using 40x assay mix per assay)	0.313 µL (using 40x assay mix per assay)	900 nM primer, 200 nM probe
Template DNA	0.8 µL	No more than 2.23 µL	No more than 4.45 µL	No more than 11.88 µL	As required
Water	-	To 5 µL	To 10 µL	To 25 µL	-

Table 3. Example of a multiplex (duplex) reaction set-up.

5. Place the reaction tubes/plates in a qPCR instrument and run the desired protocol for either end-point genotyping (Table 4 and Table 5) or qPCR (Table 6).

Step	Temperature	Time	Number of cycles
1	95 °C	15 minutes	1
2*	95 °C	15 seconds	30
	60 °C	1 minute	
3	Read		

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

Step	Temperature	Time	Number of cycles
1	95 °C	15 seconds	5
	60 °C	1 minute	
2	Read		

Table 5. Guide for end-point genotyping “recycling” protocol.

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

Table 6. 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 visit our BHQ Probe Master Mix webpage or contact techsupport@lgcgroup.com

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