BHQ Probe Master Mix quick guide

1. Thaw all components on ice, and set up all reactions on ice.
2. Prepare stock (200 µM) oligonucleotides by multiplying the nmol amount (e.g. 14.2 nM) by 10 (14.2 x 10 = 142) and divide this number by 2. This is the volume of diluent, in µL, (71 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 stock concentration	Volume	Working stock concentration
200 µM primer (each)	18 µL	36 µM	18 µL	72 µM
200 µM probe (each)	4 µL	8 µM	4 µL	16 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. For 40x assay mix, this will allow for sufficient volume to run a duplex for both qPCR and genotyping. For 80x assay mix, this will allow for sufficient volume to run a singleplex for both qPCR and genotyping. For further multiplexing, a more concentrated stock than 200 µM will be required.

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	Variable*	Variable*	Variable*	As required
Water	-	To 5 µL	To 10 µL	To 25 µL	-

Table 2. Example of a singleplex reaction set-up.
* For template DNA, use as much sample as needed, up to the maximum allowed by the reaction volume. Please note that too much DNA can have a negative impact on PCR.

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 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)	900 nM each primer, 200 nM each probe
Template DNA	0.8 µL	Variable*	Variable*	Variable*	As required
Water	-	To 5 µL	To 10 µL	To 25 µL	-

Table 3. Example of a multiplex (duplex) reaction set-up.
* For template DNA, use as much sample as needed, up to the maximum allowed by the reaction volume. Please note that too much DNA can have a negative impact on PCR.

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 T_m 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 T_m 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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