BHQ Probe Master Mix user manual

For research use only. Not for use in diagnostic procedures.

Contents

1. Introduction

BHQ® Probe Master Mix is a convenient 2x mix for endpoint probe-based genotyping, and contains all required components except DNA template, water and a probe-based assay. BHQ Probe Master Mix can be used with the genomic DNA target of your choice and any probe-based assay e.g. BHQ. BHQ Probe Master Mix is compatible with most qPCR instruments and FRET-capable plate readers.

2. Chemistry overview and key features

BHQ Probe Master Mix has been optimised for used with BHQ and BHQplus® Probes, but can be used with any probe-based genotyping assay. BHQ Probes feature a fluorophore and corresponding BHQ quenching dye. Selection of the appropriate probe type, fluorophore, and BHQ quenching dye is dependent upon your instrument, probe length, and assay type. BHQplus Probes are shortened probes that have been optimised for use with SNP genotyping.

To learn more, please visit www.biosearchtech.com/products/qpcr-and-snp-genotyping/bhqplus-probes or for help designing your probe, please visit www.biosearchtech.com/realtimedesign.

BHQ Probe Master Mix is available in a range of pack sizes from 1.25 mL up to 200 mL. It can also be supplied as no ROX, low ROX or standard ROX formulations. Product codes are detailed in Table 1.

Description	Volume	Product code
2x BHQ Probe Master Mix, No ROX	1.25 mL	KBS-1040-001
	2.5 mL	KBS-1040-002
	5 mL	KBS-1040-003
	10 mL	KBS-1040-004
	25 mL	KBS-1040-005
	200 mL	KBS-1040-006
2x BHQ Probe Master Mix, Low ROX	1.25 mL	KBS-1040-011
	2.5 mL	KBS-1040-012
	5 mL	KBS-1040-013
	10 mL	KBS-1040-014
	25 mL	KBS-1040-015
	200 mL	KBS-1040-016
2x BHQ Probe Master Mix, Standard ROX	1.25 mL	KBS-1040-021
	2.5 mL	KBS-1040-022
	5 mL	KBS-1040-023
	10 mL	KBS-1040-024
	25 mL	KBS-1040-025
	200 mL	KBS-1040-026

Table 1. BHQ Probe Master Mix formulations, pack sizes and product codes.

BHQ Probe Master Mix is supplied at 2x concentration and contains:

- KlearTaq™ Hot Start DNA polymerase, a purified DNA polymerase. The enzyme is inactive at room temperature, so reactions can be set up on the bench top. The enzyme is activated during thermal cycling and requires a 15 minute initial activation stage (95 ºC).
- Optimised components including buffer and dNTPs designed to provide consistent and accurate genotypes. High call rates can be obtained from crudely extracted plant and animal PCR templates over a broad range of final reaction volumes. dTTP is used in our BHQ Probe Master Mix as we have found that it improves reaction sensitivity and efficiency when compared to mixes containing dUTP.
- Where applicable - ROX™ may be included as a passive internal reference dye for precise data analysis (contained within the BHQ Probe Master Mix for Low ROX and Standard ROX formulations).

Key features of BHQ Probe Master Mix include:

- Specifically formulated for low-volume endpoint fluorescent detection for probe-based genotyping on crudely extracted samples
- Discrete clusters and high call rates for accurate and reproducible allelic discrimination
- Optimised to pair with BHQ Probes across a range of challenging animal and plant sample and template types.

3. Storage conditions

BHQ Probe Master Mix can be safely stored for one week at 4 ºC in a refrigerator, and one year at -20 ºC or below. If the BHQ Probe Master Mix is divided into aliquots, it is recommended that the tubes used are light-protective. Frequent freezing and thawing should be avoided for optimal performance of the mix.

4. Customer requirements

- Nuclease-free water
- Probe-based assay e.g. BHQplus
- Template DNA
- PCR plate/Array Tape®

5. Before you start

- Order probes and primers for your assay. For optimal results we recommend BHQ or BHQplus Probes. To save time, we recommend purchasing ValuMix for SNP genotyping which delivers an optimised mix of primers and probes for your SNP genotyping assay. To learn more go to: www.biosearchtech.com/products/qpcr-and-snp-genotyping/bhqplus-probes.
- Prepare DNA samples – ensure that DNA is at the appropriate concentration for the genome size of your organism. BHQ Probes and primers will function well with 5-50 ng/µL of high quality DNA per reaction (based on human genome size).
- Prepare a primer-probe mix. Guidelines on how to approach this for endpoint genotyping with BHQ Probes and primers can be found in Appendix 1; for illustrative purposes a 20x working primer-probe mix of the 2 probes and 2 primers is prepared.
- Thaw and vortex BHQ Probe Master Mix and the prepared primer-probe mix.
- If DNA is to be dried down into the Array Tape on a Nexar system with two drying heads, each drying head should be set at 75 ºC for 42,000 ms on each array. These conditions will need to be optimised if using a Nexar with greater or fewer than two DNA drying heads.

6. Protocol

BHQ Probe Master Mix is supplied at 2x concentration and should be diluted to 1x concentration in the final reaction. A typical reaction set-up should include BHQ Probe Master Mix, a probe-based assay, and template DNA. Recommended total reaction volumes are:

- 10-25 µL for 96-well plates
- 3-5 µL for 384-well plates
- 1-3 µL for 1536-well plates
- 0.8-1.6 µL in Array Tape

BHQ Probe Master Mix can be dispensed at 1x or 2x concentration, depending on the format of the DNA template (See Section 7.2, Table 2).

6.1 Instrumentation

Reactions using BHQ Probe Master Mix can be run on any standard PCR block or qPCR instruments, and read on most qPCR instruments and FRET-capable plate readers. LGC has validated BHQ Probe Master Mix on the following systems:

- Nexar® In-Line Liquid Handling and Assay Processing System (Nexar System)
- IntelliQube® instrument using Array Tape®
- CFX384 Touch™ Real-Time PCR Detection System

BHQ Probe Master Mix is also compatible with SNPline™ Lite, SNPline XL platforms and Araya® In-line Fluorescence Detection System.

6.2a Preparing the genotyping mix

Prior to dispensing, BHQ Probe Master Mix should be combined with your chosen probe-based assay (i.e. the working primer-probe mix detailed in Section 5) to create a 'genotyping mix'. Do not attempt to pipette primer-probe mix individually into each well. As a guide, Table 2 illustrates preparation of the genotyping mix for a 96-well plate, using 10 µL reactions, and including a 10 % excess.

	Concentration (x)	Volume for 1x 96-well plate (µL)	Volume for 1x 96-well plate inclusive of 10 % excess
Primer-probe mix	20x	48	52.8
BHQ Probe Master Mix	2x	480	528

Table 2. Preparation of genotyping mix for 1x 96-well plate of 10 µL reactions. This genotyping mix should then be combined with DNA in the final reaction (as detailed in Section 6.3) to ensure all components are at the required final concentration. Calculated as follows: i) Total reaction volume: 96 x 10 µL reactions = 960 µL; ii) Volume of 20x primer-probe mix required: 960 µL/20 = 48 µL; iii) Volume of 2x BHQ Probe Master Mix required: 960 µL/2 = 480 µL.

Please note: exact volume requirements for Array Tape will be calculated by the Nexar or IntelliQube software depending on your dispensing format.

6.2b Preparing the genotyping mix with ValuMix Assays for SNP genotyping

LGC supplies ValuMix Assays for SNP genotyping that consist of two BHQplus Probes and two primers combined within a single tube. This formulation minimises pipetting error and simplifies your protocol by providing mixed oligonucleotides ready for SNP genotyping. These are provided in the same 4.5:1 primer:probe ratio detailed in Appendix 1, and can also be prepared in a 20x mix.

If using ValuMix Assays, the genotyping mix for a 96-well plate should be prepared as detailed in Section 6.2a, Table 2.

To order ValuMix Assays for SNP genotyping, please visit: https://www.biosearchtech.com/products/qpcr-and-snp-genotyping/valumix-assays-for-snp-genotyping

6.3 Dispensing the genotyping mix

After preparation of the genotyping mix (Section 6.2a and b), the final reactions can be dispensed. As a guide, Table 3 illustrates the preparation of final reactions for a 10 µL reaction volume.

	Volume required per well (µL)
Genotyping mix	5.5
DNA	4.5
Total reaction volume	10

Table 3. Preparation of final reactions. This table illustrates the volumes of genotyping mix and DNA that should be dispensed for a 10 µL final reaction volume. Please note that if DNA is dried down, the reaction volume should be made up with nuclease-free water to ensure all components are at the required final concentration.

6.4 Thermal cycling

Table 4 illustrates an example thermal cycle program that can be used when performing reactions with BHQ Probe Master Mix. Step 1, the 95 ºC hot start, is an essential step for activation of the *Taq* polymerase. Step 2 can be optimised as required.

Step	Temperature (ºC)	Time	Number of cycles
1	95	15 min	1 cycle
2	95	15 sec	40 cycles
	60	60 sec	

Table 4. An example PCR thermal cycle program for use with BHQ Probe Master Mix.

6.5 Reading completed reactions

Completed reactions should be read on an appropriate FRET-capable reader. Please refer to the information supplied with your hydrolysis probe(s) to determine the appropriate read settings for your completed reactions. The passive reference dye ROX can be read using the excitation and emission spectra detailed in Table 5. Reactions run on the Nexar system can be read on the Araya Fluorescence Detection System using standard genotyping conditions.

Fluorophore	Excitation (nm)	Emission (nm)
ROX	575	610

Table 5. Excitation and emission wavelengths for the fluorophore ROX.

Endpoint genotyping data should be plotted in a cluster plot format; values for one fluorophore (e.g. probe X) should be plotted on the X axis and values for the other fluorophore (e.g. probe Y) should be plotted on the Y axis. Please refer to the information supplied with your hydrolysis probe(s) to determine which fluorophore corresponds to which allele. Values can be normalised using the ROX values for each well (if BHQ Probe Master Mix containing ROX has been used), thus removing the variable of liquid volume and leading to tighter clustering of data points. Figure 1 illustrates an example genotyping cluster plot.

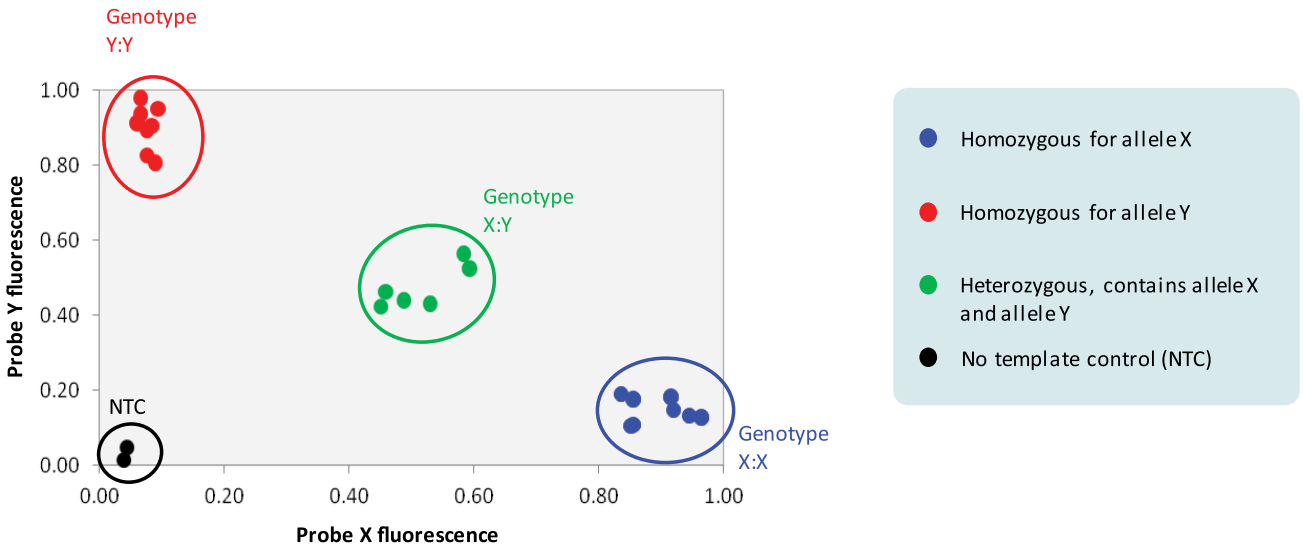

Figure 1. A typical genotyping cluster plot. Each data point represents the fluorescent signal of an individual DNA sample. Samples of the same genotype will have generated similar levels of fluorescence and will therefore cluster together on the plot.

7. Ordering information

BHQ Probe Master Mix is available in Low ROX, Standard ROX, and No ROX formulations. Please visit our website (www.lgcgroup.com/BHQMastermix) to determine the appropriate ROX level for your instrument.'

Description	Volume	Product code
2x BHQ Probe Master Mix, No ROX	1.25 mL	KBS-1040-001
	2.5 mL	KBS-1040-002
	5 mL	KBS-1040-003
	10 mL	KBS-1040-004
	25 mL	KBS-1040-005
	200 mL	KBS-1040-006
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2x BHQ Probe Master Mix, Standard ROX	1.25 mL	KBS-1040-021
	2.5 mL	KBS-1040-022
	5 mL	KBS-1040-023
	10 mL	KBS-1040-024
	25 mL	KBS-1040-025
	200 mL	KBS-1040-026

Table 6. BHQ Probe Master Mix formulations, pack sizes and product codes.

8. Obtaining support:

If you have any queries regarding BHQ Probe Master Mix, please do not hesitate to contact our technical support team who will be happy to help (tech.support@lgcgroup.com).

Appendix 1: Preparing a primer-probe mix using BHQplus Probes

This worked example assumes the following concentrations of primers and probes in the final reaction:

	Concentration required in final reaction	
Component	nM	µM
Forward primer	900	0.9
Reverse primer	900	0.9
Probe X	200	0.2
Probe Y	200	0.2

Table A1. Desired final concentrations of BHQplus primers and probes. These are suggested concentrations, and can be adjusted to suit your experiment as required.

Please note: LGC supplies ValuMix Assays for SNP genotyping that simplifies this protocol - see Section 6.2b for more details.

Step 1: Decide on a desired concentration for the primer-probe mix

The primer-probe mix for endpoint genotyping using BHQ or BHQplus Probes will include the forward and reverse primers, plus two BHQplus or BHQ Probes. In this worked example, a 20x primer-probe mix is used to illustrate the process, but this concentration can be adjusted to fit with your laboratory procedures.

Step 2: Hydrate the primers and probes

Primers and BHQ/BHQplus Probes are received dried down, and require hydration with Te buffer (10 mM Tris•Cl/0.1 mM EDTA), pH 8, or DNase-free water. In this worked example, primers and probes are hydrated to 100 µM stocks.

Each tube will have a 'total nmol' value printed on the label. To calculate the volume of buffer required:

- Convert the total nmol value into µmol (divide by 1000)
  - 35 nmol: 35 ÷ 1000 = 0.035 µmol
- Divide the µmol that you have by the concentration that you require
  - 0.035 µmol ÷ 100 µmol/L = 0.00035
  - The µmol units cancel each other out, giving the volume in litres (L) required to hydrate the primer or probe
- Convert the value in litres to microlitres
  - 0.0035 L x 1000 µL/L = 0.35 mL
  - 0.35 mL x 1000 = 350 µL buffer required to rehydrate the primer or probe to a 100 µM stock.

Table A2 illustrates the volumes of buffer required to hydrate a set of BHQ Probes and primers.

	Dry mass (nmol)*	Desired concentration (µM)**	Volume of buffer required to hydrate (µL)
Forward primer	35	100	350
Reverse primer	41	100	410
Probe X	42	100	420
Probe Y	38	100	380

Table A2. Hydration of primers and probes. *The dry mass (nmol) values are examples only – the nmol values on your tubes should be used. **The desired concentration can be adjusted as required – 100 µM has been used in this worked example.

When hydrating primers and probes:

- Spin down the tube(s) containing the dry pellet to ensure that the pellet is at the bottom of the tube.
- Add the required volume of buffer and vortex thoroughly for a minimum of 30 seconds
- Repeat the vortex step until no particulates are observed at the bottom of the tube

Step 3: Combine the hydrated primers and probes into a primer-probe mix

The primer-probe mix is prepared using the hydrated primer and probe stocks prepared in Step 2. Table A3 illustrates how to prepare 300 µL of 20x primer-probe mix. Please note: the volume to be prepared and the concentration of primer-probe mix can be adjusted as required. Table A4 illustrates the concentration of primers and probes required in the final reaction and in the primer-probe mix.

Component	Stock concentration (µM)	Desired concentration (µM)*	Volume (µL)
Forward primer	100	18	54
Reverse Primer	100	18	54
Probe X	100	4	12
Probe Y	100	4	12
Te			168
Total volume			300

Table A3. Preparation of a 20x primer-probe mix. This table illustrates how to prepare 300 µL of 20x primer-probe mix, using primer and probe stocks that have been prepared at 100 µM. *Desired concentration is calculated based on the final concentration required in the reaction, and the chosen concentration (i.e. 20x) of the primer-probe mix (see Table A4).

Component	Concentration required in final reaction (µM)	Concentration required in 20x primer-probe mix (µM)
Forward primer	0.9	18
Reverse primer	0.9	18
Probe X	0.2	4
Probe Y	0.2	4

Table A4. The concentration of the primers and probes required in the final reaction and in a 20x primer-probe mix.

Selecting the appropriate BHQ Probe Master Mix volume to go with your ValuMix Assay

ValuMix for SNP genotyping (probe concentration)	ValuMix product code	BHQ Probe Master Mix minimum kit volume	BHQ Probe Master Mix product code
2 nmol	FTBP-2 (FAM/TET) FCBP-2 (FAM/CAL Fluor Orange 560) FGBP-2 (FAM/CAL Fluor Gold 540)	5 mL	KBS-1040-003 No ROX KBS-1040-013 Low ROX KBS-1040-023 Standard ROX
5 nmol	FTBP-5 (FAM/TET) FCBP-5 (FAM/CAL Fluor Orange 560) FGBP-5 (FAM/CAL Fluor Gold 540)	25 mL	KBS-1040-005 No ROX KBS-1040-015 Low ROX KBS-1040-025 Standard ROX
12 nmol	FTBP-12 (FAM/TET) FCBP-12 (CAL Fluor Orange 560) FGBP-12 (CAL Fluor Gold 540)	200 mL	KBS-1040-006 No ROX KBS-1040-016 Low ROX KBS-1040-026 Standard ROX

Table A5. Refer to the table above to select the appropriate probe/dye combination for your ValuMix Assay with the recommended volume of BHQ Probe Master Mix.