KASP genotyping trial kit user guide

extraction • sequencing • genotyping • extraction • sequencing • genotyping • extraction • sequencing • genotyping

Contents of this guide

1. Introduction

The KASP™ genotyping trial kit is supplied as an introduction to the use of KASP chemistry. It enables the user to trial the chemistry in their own laboratory using existing equipment.

In general any qPCR instrument or plate reader that is capable of reading FRET at the wavelengths in Table 1 should be compatible with KASP.

KASP has been validated on a wide range of qPCR instruments including:

- ABI 7500
- ABI 7300
- ABI 7900
- ABI ViiA7
- Roche LC480
- Agilent Mx3000P / 3005P
- illumina EcoRT
- BIO-RAD CFX

Please visit our website www.lgcgroup.com/mastermixcheck to view a full list of the instruments that have been validated for KASP to date, to access our instrument-specific manuals, and to determine the correct version of KASP Master mix for your machine.

2. Kit contents

This kit contains a KASP genotyping assay comprising of Assay mix, Master mix and DNA:

1. 1 x 96-well microtitre plate containing 33 DNA samples (and 3 no-template controls) pre-diluted to concentration range appropriate for KASP genotyping reactions.
2. 1 x 500 µL tube of KASP Master mix at 2x concentration (sufficient for 100 reactions at 10 µL).
3. 1 x tube of KASP Assay mix (at 72x concentration).

Customer requirements

1. FRET-capable plate reader (see Table 1 for details of the excitation and emission values of the fluorophores)
2. One empty PCR plate of the type intended for use in the customer's genotyping process
3. PCR grade water
4. Optically clear seals.

Fluorophore	Excitation (nm)	Emission (nm)
FAM	485	520
HEX	535	556
ROX	575	610

Table 1: Fluorophores used in the KASP chemistry and their respective excitation and emission wavelengths.

3. Experimental procedure for performing KASP genotyping reactions

3.1 KASP genotyping reactions

The following reagents are used to prepare the KASP reaction:

- 2x KASP Master mix
- 72x KASP Assay mix
- DNA samples (sample DNA is provided at the correct concentration for using at a 2x dilution)
- Water to be added if necessary to make the final reaction volume.

3.2 Kit assembly

A 5 µL total reaction volume is required for 384-well plates and a 10 µL total reaction volume for 96-well plates.

Table 2 is an example of how to construct 60 reactions for KASP for both 384- and 96-well plate formats. This will enable you to make enough genotyping mix for the 33 samples and 3 no template controls (NTCs), with an additional spare volume.

3.3 KASP genotyping mix assembly

Component	Wet DNA method (µL) for 60 reactions	
Plate format	384-well plate	96-well plate
2x KASP Master mix	150	300
Assay mix	4.2	8.4
Water	N / A	N / A
Total reaction volume	5	10
Total	300	600

Table 2: An example of KASP reaction assembly

3.4 Experimental procedure

1. Thaw the KASP Master mix and KASP Assay mix tubes. Vortex each tube, and store on ice.
2. Thaw the DNA plate. Briefly vortex and spin down the plate.
3. Use a single- or multi-channel pipette to transfer DNA to the empty reaction plate (supplied by customer).
   - **For a 96-well plate: transfer 5 µL of the DNA provided to each well.**
   - **For a 384-well plate: transfer 2.5 µL of the DNA provided to each well.**
4. Prepare the genotyping mix (2x KASP Master mix plus Assay mix) as outlined in Table 2. Add the required amount of genotyping mix to each DNA sample in the reaction plate. A single- or multi-channel pipette can be used for this procedure.
   - **For a 96-well plate: add 5 µL of the prepared genotyping mix to each DNA sample.**
   - **For a 384-well plate: add 2.5 µL of the prepared genotyping mix to each DNA sample.**
5. Seal the plate with an optically clear seal.
6. Centrifuge the plate at a minimum of 550 *x* g. Do not spin the plates at a higher speed than is recommended for your rotor.

3.5 Thermal cycling conditions

The KASP chemistry can be used with any standard thermal cycler. The thermal cycling conditions are detailed in Table 3. Please note that a two-step touchdown PCR method is used, with the elongation and annealing steps incorporated into a single step. It is recommended to cycle the reaction plates as soon as possible after the DNA and genotyping mix have been dispensed. If this is not feasible, prepared plates can be stored for up to 1 hour at 4^{O}C. Plates should be sealed with an optically clear seal immediately after dispensing to prevant any evaporation.

Step	Description	Temperature	Time	Number of cycles per step
1	Activation	94^{O}C	15 min	1
2	Denaturation	94^{O}C	20 sec	10 cycles
	Annealing / Elongation	61-55^{O}C	60 sec (drop 0.6^{O}C per cycle)	
3	Denaturation	94^{O}C	20 sec	26 cycles
	Annealing / Elongation	55^{O}C	60 sec	

Table 3: Thermal cycling conditions for the KASP chemistry

After thermal cycling, read the plate.

Please note: All plates should be read below 40^{O}C. If the plate is not read below 40^{O}C, it will not be possible to analyse the genotyping data.

3.6 Additional cycling conditions

If you have not obtained clear genotyping clusters, the plate should be thermally cycled for an additional 3 cycles and read again. Please see Table 4 for recycling conditions.

Step	Temperature	Time	Number of cycles per step
Denaturation	94°C	20 sec	3 cycles
Annealing / Elongation	57°C	60 sec	

Table 4: Conditions for further thermal cycling of the KASP chemistry

Further cycling and reading can be performed until tight genotyping clusters have been attained.

Figure 1

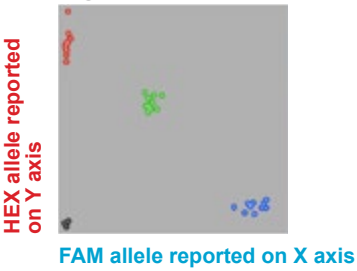

Figure 2

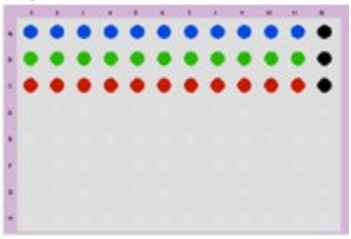

Figure 1: Example genotyping plot illustrating three clear clusters. The FAM allele is reported on the X axis (blue data points) and the HEX allele is reported on the Y axis (red data points). The green data points represent heterozygous samples and the black data points are the No Template Controls (NTCs).

Figure 2: A 96-well plate layout indicating expected genotyping results of the KASP genotyping trial kit DNA samples.

Note: This protocol pertains to all three of the trial kit part numbers. The only difference between the three kits is the concentration of the passive reference dye ROX in the KASP Master mix, which varies based upon the type of reader being used. To determine which trial kit is right for you, please contact our technical support team tech.support@lgcgroup.com or visit our website www.lgcgroup.com/mastermixcheck.

4. Ordering information

Product code	Description
KBS-1014-104	Standard Rox trial kit
KBS-1014-105	High Rox trial kit
KBS-1014-106	Low Rox trial kit

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