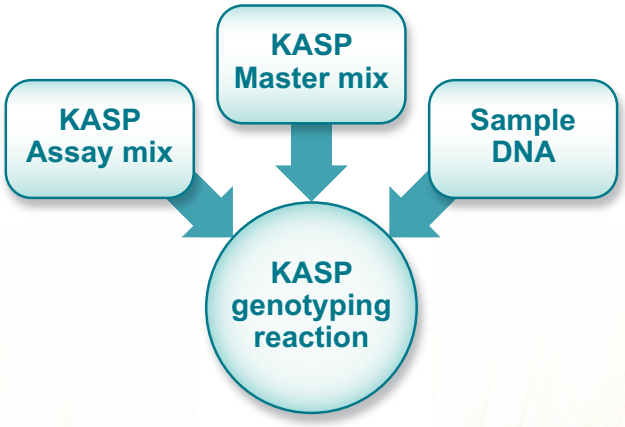
KASP genotyping

Quick start guide

This document is intended as a quick start guide to running KASP™ genotyping reactions in your laboratory. For full details on KASP, including experimental setup and data analysis, please consult our full user guide and manual (downloadable from our website).



1. Array DNA samples into the reaction plate
2. Prepare the genotyping mix
3. Dispense genotyping mix onto the reaction plate
4. Seal and centrifuge the plate
5. Run the thermal cycle
6. Read the plate and analyse the data

The full KASP user guide and manual can be downloaded from **www.lgcgroup.com/genomics**

Before you start

- KASP Assay mix should be ordered from LGC. Each KASP Assay mix is specific to the SNP or InDel that is to be targeted, and consists of two competitive allele-specific primers and one common reverse primer. Two options are available for ordering KASP Assay mix:
  - KASP-by-Design (KBD) – primers based on *in silico* design, no wet lab validation
  - KASP-on-Demand (KOD) – optimised and functionally validated in our genotyping laboratory
- Please ensure that you are using the optimal version of KASP Master mix for your instrument (visit www.lgcgroup.com/mastermixcheck). KASP Master mix is available in Low, Standard, and High ROX formulations. **Please note:** these formulations only differ in the level of ROX that they contain and are otherwise identical
- Thaw and vortex KASP Assay mix and KASP Master mix. **Please note:** KASP Master mix should be aliquotted for storage. Freeze-thaw cycles should be avoided.
- Prepare DNA samples – ensure that these are at the appropriate concentration for the genome size of your organism. Most KASP assays will function well with 5-50 ng of high quality DNA per reaction (based on human genome size).

1. Array DNA samples into the reaction plate

DNA samples should be arrayed into a 96-well or 384-well PCR plate. No-template controls (NTCs) should be included on each plate. Arrayed DNA can be dried down or stored in hydrated form if genotyping reactions are not to be run straight away.

Please note: KASP genotyping can also be run in 1536-well plates when used in conjunction with the LGC SNPline instrumentation and appropriate KASP Master mix (1536 formulation).

2. Prepare the KASP genotyping mix

Table 1 details the constituent reagent volumes required for preparing KASP genotyping mix for both 96-well and 384-well plates. Where the user prefers to dry down the arrayed DNA samples, the KASP Master mix must be diluted by the addition of molecular biology grade water, to bring the overall final mix concentration to 1x.

All reagents should be briefly vortexed before use. Prepare sufficient genotyping mix for the number of reactions that you intend to perform, plus an additional dead volume.

	Wet DNA method		Dry DNA method	
	96-well plate (µL per well)	384-well plate (µL per well)	96-well plate (µL per well)	384-well plate (µL per well)
DNA	5	2.5	n/a	n/a
2x KASP Master mix	5	2.5	5	2.5
KASP Assay mix	0.14	0.07	0.14	0.07
Water	n/a	n/a	5	2.5
Total reaction volume	10	5	10	5

Table 1. Constituent reagent volumes for KASP genotyping reactions.

For any queries on this manual or running KASP reactions in your laboratory please contact:
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3. Dispense genotyping mix onto the reaction plate

Add the required amount of genotyping mix to each DNA sample in the reaction plate using a pipette or dispensing robot.

WET DNA:

For 96-well plates: 5 µL genotyping mix per sample well.
For 384-well plates: 2.5 µL genotyping mix per sample well.

DRY DNA:

For 96-well plates: 10 µL genotyping mix per sample well.
For 384-well plates: 5 µL genotyping mix per sample well.

4. Seal and centrifuge the plate

Seal the plate with an optically clear seal. Centrifuge the plate at ≥550 *x* g.

5. Run the thermal cycle

KASP chemistry can be used with any standard thermal cycler. KASP uses a set of thermal cycling conditions comprised of two temperature steps, rather than the more traditional three steps.

Run the KASP thermal protocol as outlined in Table 2.

Step	Description	Temperature	Time	Number of cycles per step
1	Activation	94°C	15 minutes	1 cycle
2	Denaturation	94°C	20 seconds	10 cycles
	Annealing / Elongation	61-55°C	60 seconds (drop 0.6°C per cycle)	
3	Denaturation	94°C	20 seconds	26 cycles
	Annealing / Elongation	55°C	60 seconds	

Table 2. Thermal cycle conditions for KASP genotyping reactions.

6. Read the plate and analyse the data

After completion of the thermal cycle, read the reaction plate in a FRET-capable plate reader.

Please note: all plates should be read below 40°C. If read above this temperature, it will not be possible to analyse the genotyping data.

To analyse the data, import it into a genotype cluster analysis software package such as KlusterCaller™ (LGC) (www.lgcgroup.com/software). The data can be plotted and genotyping clusters analysed as shown in Figure 1.

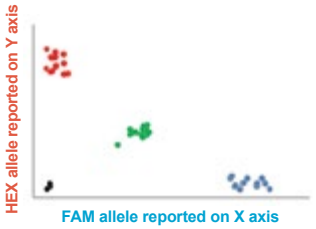

Figure 1. Example cluster plot for KASP genotyping data.

If sufficiently defined genotype clusters are not obtained after the initial KASP thermal cycle, the plate should be thermally cycled for an additional three cycles using the conditions detailed in Table 3. The reaction plate should then be re-read in the FRET-capable plate reader and results analysed.

Step	Temperature	Time	Number of cycles
Denature	94°C	20 seconds	3 cycles
Annealing / Elongation	57°C	60 seconds	

Table 3. Temperature conditions for recycling of KASP reaction plates.

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Further thermal cycling and plate reading can be performed until defined genotype clusters have been attained.

Please note: the tubes of $MgCl_2$ and DMSO that are supplied with the KASP Master mix are only intended for use during troubleshooting of assays with extremely high / low GC content. Please contact our technical support team if you require any assistance (contact details below).

Ordering information

Please ensure that you are using the optimal version of KASP Master mix for your instrument (visit www.lgcgroup.com/mastermixcheck).

Cat. No.	Kit	Starting material
KBS-1016-001	KASP V4.0 2X Master mix 96/384, Std Rox,	500 x 10 µL reactions (2.5 mL)
KBS-1016-002	KASP V4.0 2X Master mix 96/384, Std Rox,	5,000 x 10 µL reactions (25 mL)
KBS-1016-003	KASP V4.0 2X Master mix 96/384, Std Rox,	50,000 x 10 µL reactions (250 mL)
KBS-1016-016	KASP V4.0 2X Master mix 96/384, Low Rox,	500 x 10 µL reactions (2.5 mL)
KBS-1016-017	KASP V4.0 2X Master mix 96/384, Low Rox,	5,000 x 10 µL reactions (25 mL)
KBS-1016-021	KASP V4.0 2X Master mix 96/384, High Rox,	500 x 10 µL reactions (2.5 mL)
KBS-1016-022	KASP V4.0 2X Master mix 96/384, High Rox,	5,000 x 10 µL reactions (25 mL)
KBS-1016-023	KASP V4.0 2X Master mix 96/384, High Rox	50,000 x 10 µL reactions (250 mL)

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