

KASP genotyping – quick guide

This document is intended as a quick guide to running KASP™ end-point genotyping reactions in your laboratory. For full details on KASP, including experimental setup and data analysis, please consult our [full user guide](#).

Before you start

- KASP Assay Mix should be ordered from LGC, Biosearch Technologies™. 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-TF Master Mix for your instrument (visit: www2.lgcgroup.com/KASP_protocols_instruments). KASP-TF 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-TF Master Mix. Please note: KASP-TF 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).

Technical support

If you have technical queries regarding the running of KASP end-point genotyping in your laboratory, please contact techsupport@lgcgroup.com.

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.

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.

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-TF 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. Where the user prefers to dry down the arrayed DNA samples, the KASP-TF Master Mix must be diluted by the addition of molecular biology grade water, to bring the overall final mix concentration to 1x.

Please note: the tubes of MgCl₂ and DMSO that are supplied with KASP-TF Master Mix are only intended for use during troubleshooting of assays with extremely high/low GC content.

3. Dispense the 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

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4. Seal and centrifuge the plate

Seal the plate with an optically clear seal. Centrifuge the plate at $\geq 550 \times g$.

5. Run the thermal cycle

KASP chemistry can be used with any standard thermal cycler. 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 cycling 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™](#), from Biosearch Technologies. The data can be plotted and genotyping clusters analysed as shown in Figure 1.

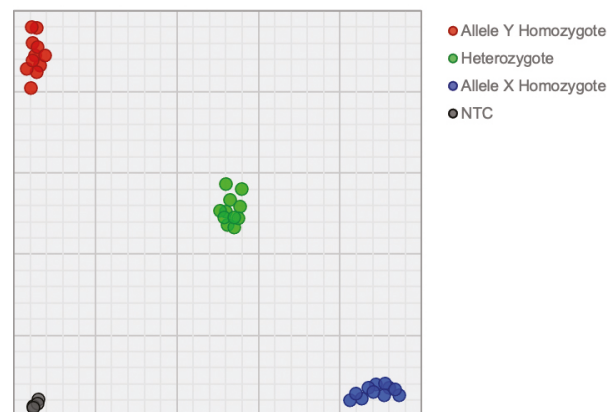


Figure 1. Example cluster plot for KASP genotyping data

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

Ordering information

Part Number	Description
KBS-1050-101	KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (2.5 mL)
KBS-1050-102	KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (25 mL)
KBS-1050-103	KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (250 mL) (10 x 25 mL)
KBS-1050-121	KASP-TF V4.0 2X Master Mix 96/384, Low ROX (2.5 mL)
KBS-1050-122	KASP-TF V4.0 2X Master Mix 96/384, Low ROX (25 mL)
KBS-1050-123	KASP-TF V4.0 2X Master Mix 96/384, Low ROX (250 mL) (10 x 25 mL)
KBS-1050-131	KASP-TF V4.0 2X Master Mix 96/384, High ROX (2.5 mL)
KBS-1050-132	KASP-TF V4.0 2X Master Mix 96/384, High ROX (25 mL)
KBS-1050-133	KASP-TF V4.0 2X Master Mix 96/384, High ROX (250 mL) (10 x 25 mL)
KBS-1050-111	KASP-TF V4.0 2X Master Mix 1536, Standard ROX (2.5 mL)
KBS-1050-112	KASP-TF V4.0 2X Master Mix 1536, Standard ROX (25 mL)
KBS-1050-113	KASP-TF V4.0 2X Master Mix 1536, Standard ROX (250 mL) (10 x 25 mL)

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