KASP Array Tape Master mix

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

Contents

1. Product description and specification

KASP Array Tape Master mix has been optimised for high-throughput single nucleotide polymorphism (SNP) and Insertion/Deletion (InDel) genotyping in Array Tape®. This 2X mix has been designed specifically for use with Array Tape on the Nexar® In-Line Liquid Handling and Assay Processing System (Nexar® System) and the IntelliQube® instrument.

KASP Array Tape Master mix is available in three pack sizes, as detailed in Table 1.

Product code	Pack size	Description
KBS-1030-001	2.5 mL	KASP Array Tape 2X Master mix, Standard ROX
KBS-1030-002	25 mL	KASP Array Tape 2X Master mix, Standard ROX
KBS-1030-003	250 mL	KASP Array Tape 2X Master mix, Standard ROX

Table 1. Product codes and pack sizes for KASP Array Tape Master mix.

2. Kit contents and storage

i. KASP Array Tape Master mix – supplied at 2X concentration. Contains FAM™ and HEX™ specific FRET cassettes, KASP *Taq*, dNTPs, salts, and buffer.

ii. $MgCl_2$ – 50 mM, for optimisation of assays in particularly A/T-rich DNA regions.

iii. DMSO – for optimisation of assays in particularly G/C-rich DNA regions.

KASP Array Tape Master mix can be safely stored for 1 week at 4°C, one year at -20°C, or indefinitely at -80°C. KASP Array Tape Master mix should be aliquoted for storage, and use of light-protective tubes is recommended. Frequent freezing and thawing of KASP Array Tape Master mix is not recommended.

3. 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.
- Thaw and vortex KASP Assay mix and KASP Master mix.
- 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).
- 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°C for 42000 ms on each array. These conditions will need to be optimised if using a Nexar with greater or fewer than two DNA drying heads.

4. Prepare the KASP Genotyping mix

KASP Array Tape Master mix can be dispensed at 1X or 2X concentration, depending on the format of the DNA template, as detailed in Table 2.

DNA format	Instrument	KASP Array Tape Master mix	Recommended total reaction volume
Wet DNA	IntelliQube® instrument Nexar® system	Dispense at 2X concentration (as supplied)	1.6 µL (See Table 3)
Dry DNA	Nexar® system in conjunction with the Array Tape® Sample Dryer (i.e. DNA samples dried down prior to KASP Genotyping mix dispense)	Dispense at 1X concentration. N.B. KASP Array Tape 2X Master mix must be diluted by the addition of molecular biology grade water, to bring the overall final mix concentration to 1X.	0.8 µL (See Table 4)

Table 2. Format for dispensing KASP Array Tape Master mix for both wet and dried down DNA samples.

Prior to dispensing, KASP Array Tape Master mix must be combined with KASP Assay mix according to the ratios detailed in Tables 3 and 4 (for wet and dry DNA respectively). Exact volume requirements for each Array Tape will be calculated by the Nexar or IntelliQube software depending on your dispensing format (Tables 3 and 4 are for illustration only).

In Array Tape format, KASP performs well with total reaction volumes ranging from 0.8 – 1.6 µL.

	Volume per reaction	Volume for 384 reactions (including 20% excess)
DNA sample	0.8 µL	-
KASP Array Tape Master mix 2X	0.8 µL	368.6 µL
KASP Assay mix (72x, as supplied)	0.022 µL	10.14 µL
Total reaction volume	1.6 µL	-

Table 3. Constituent reagent volumes for KASP genotyping reactions using wet DNA. KASP Array Tape Master mix should be used as supplied (2X).

	Volume per reaction	Volume for 384 reactions (including 20% excess)
DNA sample	n/a (dry DNA)	-
KASP Array Tape Master mix 1X	0.8 µL	368.6 µL
KASP Assay mix (72x, as supplied)	0.011 µL	5.07 µL
Total reaction volume	.8 µL	-

Table 4. Constituent reagent volumes for KASP genotyping reactions using dry DNA. KASP Array Tape Master mix should be diluted to 1X using molecular biology grade water.

5. Dispensing

After preparation of the KASP genotyping mix as detailed in Section 4, this should be dispensed using the Nexar® system or IntelliQube® instrument. For detailed instructions on programming the dispense steps, please refer to the appropriate instrument manual.

6. KASP cycling conditions

The KASP thermal cycling conditions detailed in Table 5 should be used when running KASP on the Nexar® System or IntelliQube® instrument.

Protocol Stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Hot start *Taq* activation	94 °C	15 minutes	x 1 cycle
Stage 2 Touchdown	94 °C	20 seconds	x 10 cycles
	65 °C (65 °C decreasing 0.8 °C per cycle to achieve a final annealing / extension temperature of 57 °C)	60 seconds	
Stage 3 Amplification	94 °C 57 °C	20 seconds 60 seconds	x 26 cycles

Table 5. KASP thermal cycling conditions for use with the Nexar® System and the IntelliQube® instrument. Please note that these conditions are optimised specifically for Array Tape and should not be used for standard PCR plates.

If sufficiently defined genotyping clusters are not obtained after 36 PCR cycles, Array Tape should be further thermally cycled in increments of 3 cycles; the recommended recycling protocol is detailed in Table 6. After each 'recycling protocol' (see Table 6), the reactions should be re-read.

Protocol Stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Amplification	94 °C	20 seconds	x 3 cycles
	57 °C	60 seconds	

Table 6. KASP further cycling conditions for use with the Nexar® System and the IntelliQube® instrument when additional thermal cycling is required.

7. Reading KASP reactions

Reactions should be read after completion of 36 cycles (as per Table 5). KASP reactions run on the Nexar® system can be read on the Araya® In-Line Fluorescence Detection System using standard genotyping conditions; there is no requirement to modify any of the read settings.

To read KASP on the IntelliQube® instrument, the fluorophores detailed in Table 7 should be selected during run setup.

Fluorophore	IntelliQube reference
ROX	Passive reference dye
FAM	X-axis
HEX (or VIC*)	Y-axis

Table 7. Fluorophores required to read completed KASP reactions on the IntelliQube instrument. *If the available dye sets contain VIC but not HEX, VIC can be used instead as the excitation and emission values for HEX and VIC are extremely similar.

KASP genotyping data should be plotted in a cluster plot format; typically FAM values are plotted on the X axis and HEX values are plotted on the Y axis. Values can be normalised using the ROX values for each well thus removing the variable of liquid volume and leading to tighter clustering of data points. Figure 1 illustrates an example genotyping cluster plot.

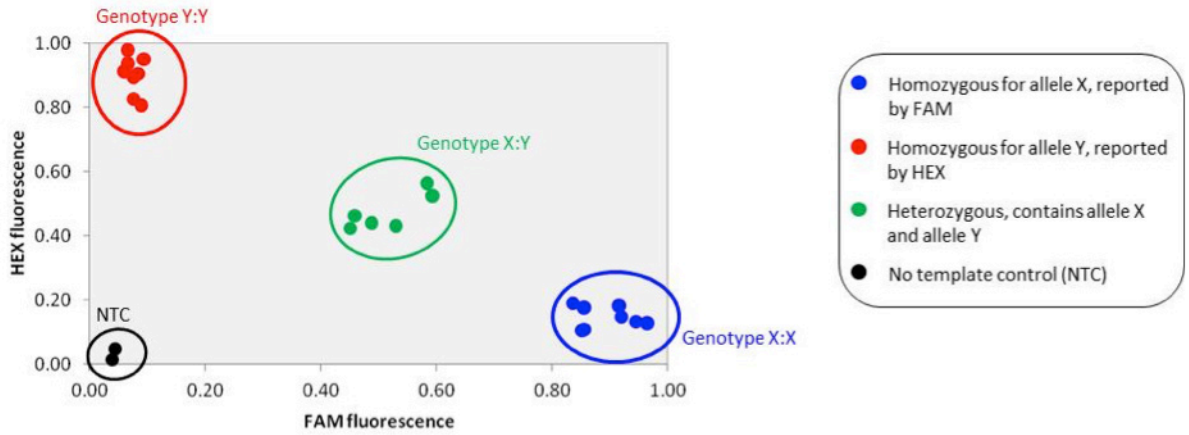

Figure 1. A typical genotyping cluster plot. Each data point represents the fluorescence signal of an individual DNA sample. Samples of the same genotype will have generated similar levels of fluorescence and will therefore cluster together on the plot.