

Manual

KASP-TF V5.0 Master Mix

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KASP-TF V5.0 Master Mix

1. Product description and specification

[KASP-TF V5.0 Master Mix](#) by LGC, Biosearch Technologies™ 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](#).

In high-throughput experiments, users typically perform thermal cycling of Array Tape using a [Hydrocycler²™](#) or a [Soellex™](#). It is possible to perform thermal cycling of Array Tape on the IntelliQube instrument; please see [Appendix A](#) for details.

KASP-TF V5.0 Master Mix is available in three pack sizes, as detailed in Table 1.

Product code	Pack size	Pack size
KBS-1050-201	2.5 mL	KASP-TF V5.0 2X Master Mix, Standard ROX
KBS-1050-202	25 mL	KASP-TF V5.0 2X Master Mix, Standard ROX
KBS-1050-204	250 mL	KASP-TF V5.0 2X Master Mix, Standard ROX
KBS-1050-203	(250 mL) (10 × 25 mL)	KASP-TF V5.0 2X Master Mix, Standard ROX

Table 1. Product codes and pack sizes for KASP-TF V5.0 Master Mix.

2. Kit contents and storage

- KASP-TF V5.0 Master Mix – supplied at 2X concentration. Contains FAM™ and HEX™ specific FRET cassettes, KASP *Taq*, dNTPs, salts, and buffer.
- MgCl₂ – 50 mM, for optimisation of assays in particularly A/T-rich DNA regions.
- DMSO – for optimisation of assays in particularly G/C-rich DNA regions.

KASP-TF V5.0 Master Mix can be safely stored for 1 week at 4 °C, one year at -20 °C, or indefinitely at -80 °C. KASP-TF V5.0 Master Mix should be aliquoted for storage, and use of light-protective tubes is recommended. Frequent freezing and thawing of KASP-TF V5.0 Master Mix is not recommended.

3. Before you start

- KASP Assay Mix should be ordered from 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.

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- Thaw and vortex KASP Assay Mix and KASP-TF V5.0 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-TF V5.0 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-TF V5.0 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-TF V5.0 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-TF V5.0 Master Mix for both wet and dried down DNA samples.

Prior to dispensing, KASP-TF V5.0 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-TF V5.0 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-TF V5.0 Master Mix should be used as supplied (2X).

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	Volume per reaction	Volume for 384 reactions (including 20% excess)
DNA sample	n/a (dry DNA)	-
KASP-TF V5.0 Master Mix 1X	0.8 µL	368.6 µL
KASP Assay Mix (72x, as supplied)	0.011 µL	5.07 µL
Total reaction volume	0.8 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 in Array Tape, using a waterbath-based high-throughput thermal cycler (Hydrocycler² or Soellex). Appendix A details the thermal cycle program to use when Array Tape is to be thermally cycled on the IntelliQube instrument.

Protocol stage	Temperature	Duration	Number of cycles for each stage
Stage 1 hot start <i>Taq</i> activation	94 °C	15 minutes	× 1 cycle
	94 °C	20 seconds	
Stage 2 touchdown	65 °C (65 °C decreasing 0.8 °C per cycle to achieve a final annealing/extension temperature of 57 °C)	60 seconds	× 10 cycles
Stage 3 amplification	94 °C	20 seconds	× 26 cycles
	57 °C	60 seconds	

Table 5. KASP thermal cycling conditions for reactions that will be read using the Nexar System or 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 three cycles; the recommended recycling protocol is detailed in Table 6. After each 'recycling protocol' (see Table 6), the reactions should be re-read.

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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 reactions that will be read using the the Nexar system or the IntelliQube instrument when additional thermal cycling is required.

Please note, if you are using the IntelliQube to perform thermal cycling, instead of a Hydrocycler or the Sollex, refer to the protocol detailed in Appendix A.

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.

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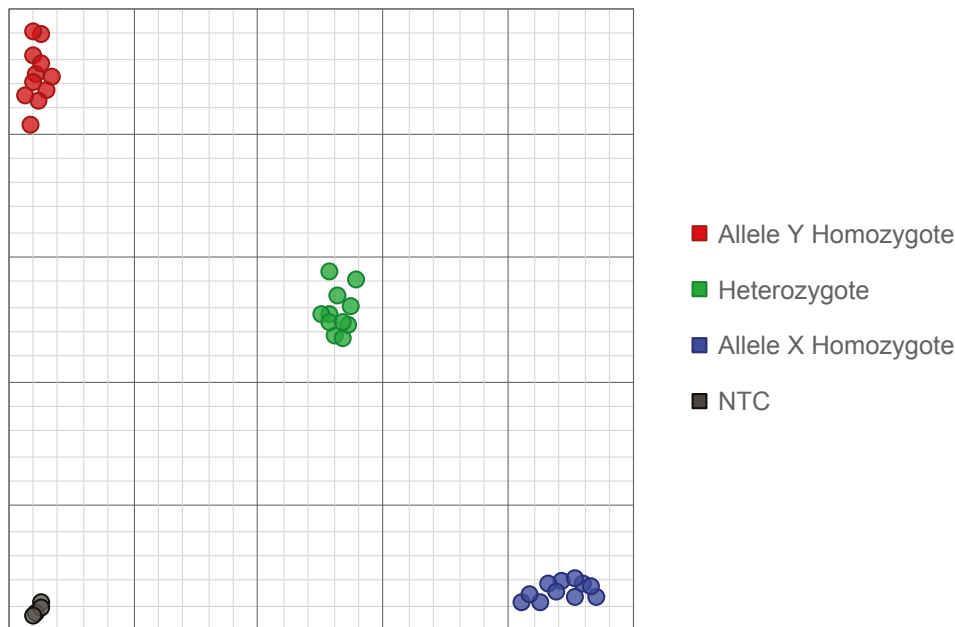


Figure 1. Example genotyping plot illustrating three clear clusters. Each data point represents the fluorescence signal of an individual DNA sample. Samples of the same genotype will have generated similar levels of fluorescence and will therefore cluster together on the plot. The FAM allele is reported 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).

If you have any queries, please contact Technical Support:

techsupport@lgcgroup.com.

Appendix A: Performing the KASP thermal cycle on the IntelliQube instrument

If the IntelliQube is being used to perform the thermal cycle, as well as to dispense and read KASP reactions in Array Tape, LGC, Biosearch Technologies recommend that one of the following thermal cycles is programmed:

- KASP IntelliQube Inline protocol: 61-55 °C touchdown (Table A1, Figure A1).
- KASP IntelliQube Inline protocol: 65-57 °C touchdown (Table A2, Figure A2).

These protocols involve a hot-start activation, a 10 PCR cycle touchdown, and then 35 PCR cycles (totalling 45 cycles, nine more than the typical KASP thermal cycle). In the third stage, a read is performed on the IntelliQube at the end of each cycle. It is not possible to carry out a traditional KASP recycling protocol in Array Tape on the IntelliQube, after the IntelliQube Inline thermal profile has been completed. Instead, performing a read every cycle in Stage 3, and running more cycles as standard, enables the most appropriate read to be selected for data analysis upon run completion.

Please note that reading the reactions every cycle does not equate to these reactions being run in real-time; the most appropriate read is selected for end-point genotyping analysis.

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Protocol stage	Temperature	Duration	Number of cycles for each stage	Read	
Stage 1 hot start Taq activation	93.5 °C	15 min (900 seconds)	× 1 cycle	No	
Stage 2 touchdown	Cycle 1	93.5 °C	20 sec	× 1 cycle	No
		61.0 °C	1 min		
	Cycle 2	93.5 °C	20 sec	× 1 cycle	No
		60.4 °C	1 min		
	Cycle 3	93.5 °C	20 sec	× 1 cycle	No
		59.8 °C	1 min		
	Cycle 4	93.5 °C	20 sec	× 1 cycle	No
		59.2 °C	1 min		
	Cycle 5	93.5 °C	20 sec	× 1 cycle	No
		58.6 °C	1 min		
	Cycle 6	93.5 °C	20 sec	× 1 cycle	No
		58.0 °C	1 min		
	Cycle 7	93.5 °C	20 sec	× 1 cycle	No
		57.4 °C	1 min		
	Cycle 8	93.5 °C	20 sec	× 1 cycle	No
		56.8 °C	1 min		
	Cycle 9	93.5 °C	20 sec	× 1 cycle	No
		56.2 °C	1 min		
	Cycle 10	93.5 °C	20 sec	× 1 cycle	No
		55.6 °C	1 min		
Stage 3 amplification	93.5 °C	20 sec	× 35 cycles	Yes	
	55.0 °C	1 min			

Table A1. KASP IntelliQube in-line thermal cycle protocol for 61-55 °C touchdown.

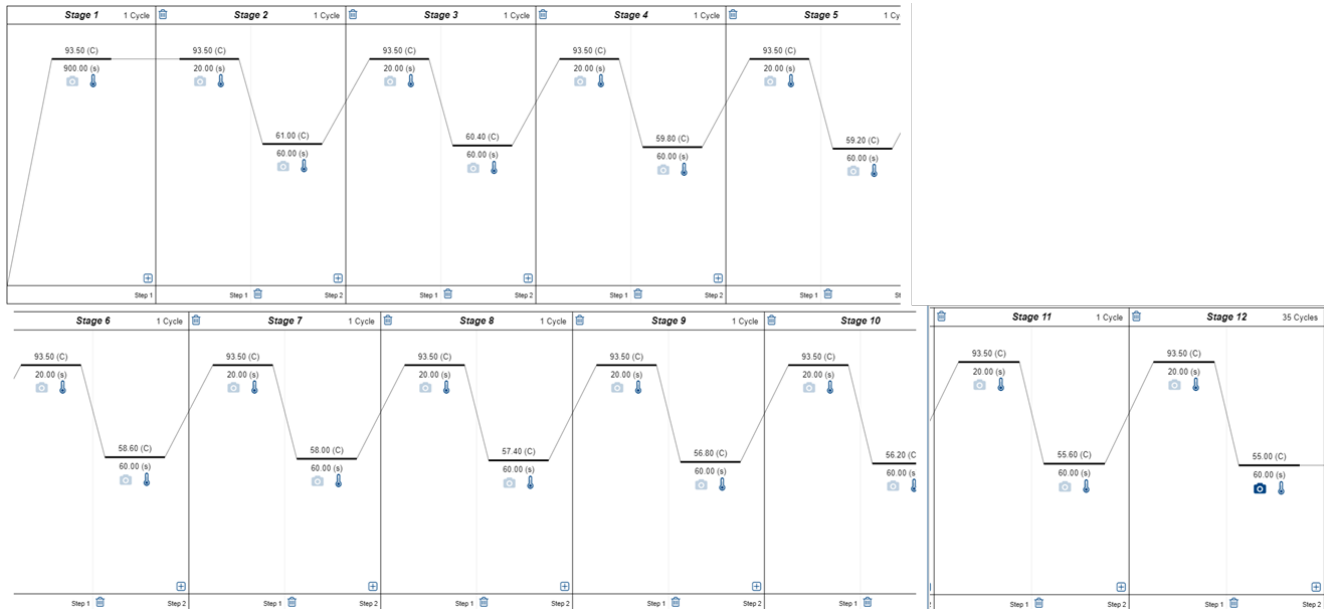


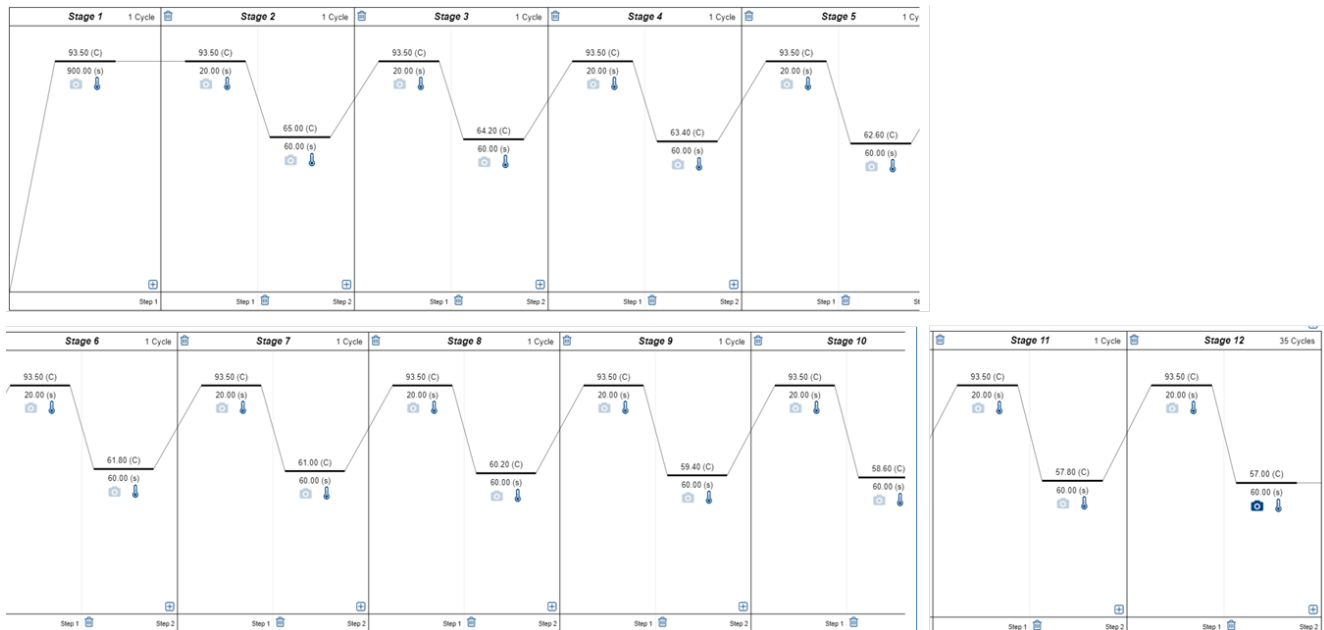
Figure A1. Screenshot of the 61-55 °C protocol when programmed in the IntelliQube software.

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Protocol stage	Temperature	Duration	Number of cycles for each stage	Read	
Stage 1 hot start <i>Taq</i> activation	93.5 °C	15 min (900 seconds)	× 1 cycle	No	
Stage 2 touchdown	Cycle 1	93.5 °C	20 sec	× 1 cycle	No
		65.0 °C	1 min		
	Cycle 2	93.5 °C	20 sec	× 1 cycle	No
		64.2 °C	1 min		
	Cycle 3	93.5 °C	20 sec	× 1 cycle	No
		63.4 °C	1 min		
	Cycle 4	93.5 °C	20 sec	× 1 cycle	No
		62.6 °C	1 min		
	Cycle 5	93.5 °C	20 sec	× 1 cycle	No
		61.8 °C	1 min		
	Cycle 6	93.5 °C	20 sec	× 1 cycle	No
		61.0 °C	1 min		
	Cycle 7	93.5 °C	20 sec	× 1 cycle	No
		60.2 °C	1 min		
	Cycle 8	93.5 °C	20 sec	× 1 cycle	No
		59.4 °C	1 min		
	Cycle 9	93.5 °C	20 sec	× 1 cycle	No
		58.6 °C	1 min		
	Cycle 10	93.5 °C	20 sec	× 1 cycle	No
		57.8 °C	1 min		
Stage 3 amplification	93.5 °C	20 sec	× 35 cycles	Yes	
	57.0 °C	1 min			

Table A2. KASP IntelliQube in-line thermal cycle protocol for 65-57 °C touchdown.



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Figure A2. Screenshot of the 61-55 °C protocol when programmed in the IntelliQube software.

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