Manual

KASP thermal cycling conditions

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

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KASP thermal cycling conditions

61-55 °C touchdown protocol

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
	61 °C (61 °C decreasing 0.6 °C per cycle to achieve final annealing/extension temperature of 55 °C)	60 seconds	
Stage 3 Amplification	94 °C	20 seconds	x 26 cycles
	55 °C	60 seconds	
Optional stage 4 (read stage for qPCR instruments only)	30 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	x 1 cycle

* Please note that stage 4 of the above program is only required if running and reading KASP™ genotyping reactions on a qPCR machine. If running the KASP thermal cycle program on a Peltier block or a Hydrocycler™, only Stages 1, 2 and 3 are needed although you must ensure that the reaction plates are cooled to <40 °C before performing the template read.

Explanation of touchdown PCR

KASP chemistry utilises a two-step touchdown PCR method, with the elongation and annealing steps incorporated into a single step. The temperature used for the annealing stage determines the specificity of the reaction and hence the ability of the primers to anneal to the DNA template. A touchdown PCR involves starting with a high annealing temperature and incrementally decreasing the annealing temperature each PCR cycle. The higher annealing temperatures in the early cycles of a touchdown ensure that only very specific base pairing will occur between the DNA and the primer, hence the first sequence to be amplified is most likely to be the sequence of interest. The annealing temperature is gradually decreased to increase the efficiency of the reaction. The regions that were originally amplified during the highly specific early touchdown cycles will be further amplified and outcompete any non-specific amplification that may occur at the lower temperatures. The standard KASP thermal cycling protocol has 10 cycles of touchdown PCR (annealing 61 °C to 55 °C, decreasing 0.6 °C per cycle), then 26 cycles of standard 2-step PCR at the lower annealing temperature (55 °C).

For any queries about running KASP reactions in your laboratory please contact the technical support team at techsupport@lgcgroup.com.

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KASP thermal cycling conditions

68-62 °C touchdown protocol

This alternative thermal cycle programme is typically used for assays with high percentage GC content.

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
	68 °C (68 °C decreasing 0.6 °C per cycle to achieve final annealing/extension temperature of 62 °C)	60 seconds	
Stage 3 Amplification	94 °C	20 seconds	x 26 cycles
	62 °C	60 seconds	
Optional stage 4 (read stage for qPCR instruments only)	30 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	x 1 cycle

* Please note that stage 4 of the above program is only required if running and reading KASP genotyping reactions on a qPCR machine. If running the KASP thermal cycle program on a Peltier block or a Hydrocycler, only Stages 1, 2 and 3 are needed although you must ensure that the reaction plates are cooled to <40 °C before performing the template read.

Explanation of touchdown PCR

KASP chemistry utilises a two-step touchdown PCR method, with the elongation and annealing steps incorporated into a single step. The temperature used for the annealing stage determines the specificity of the reaction and hence the ability of the primers to anneal to the DNA template. A touchdown PCR involves starting with a high annealing temperature and incrementally decreasing the annealing temperature each PCR cycle. The higher annealing temperatures in the early cycles of a touchdown ensure that only very specific base pairing will occur between the DNA and the primer, hence the first sequence to be amplified is most likely to be the sequence of interest. The annealing temperature is gradually decreased to increase the efficiency of the reaction. The regions that were originally amplified during the highly specific early touchdown cycles will be further amplified and outcompete any non-specific amplification that may occur at the lower temperatures.

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KASP thermal cycling conditions

2-Step 57 °C touchdown protocol

This alternative thermal cycle programme is typically used for assays with low percentage GC content.

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 Amplification	94 °C	20 seconds	x 36 cycles
	57 °C	60 seconds	
Optional stage 3 (read stage for qPCR instruments only)	30 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	x 1 cycle

* Please note that stage 3 of the above program is only required if running and reading KASP genotyping reactions on a qPCR machine. If running the KASP thermal cycle program on a Peltier block or a Hydrocycler, only Stages 1 and 2 are needed although you must ensure that the reaction plates are cooled to <40 °C before performing the template read.

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KASP thermal cycling conditions

65-57 °C touchdown protocol

This thermal cycle programme details the standard conditions for the Fluidigm platform.

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 final annealing/extension temperature of 57 °C)	60 seconds	
Stage 3 Amplification	94 °C	20 seconds	x 26 cycles
	57 °C	60 seconds	
Optional stage 4 (read stage for qPCR instruments only)	30 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	x 1 cycle

* Please note that stage 4 of the above program is only required if running and reading KASP genotyping reactions on a qPCR machine. If running the KASP thermal cycle program on a Peltier block or a Hydrocycler, only Stages 1, 2 and 3 are needed although you must ensure that the reaction plates are cooled to <40 °C before performing the template read.

Explanation of touchdown PCR

KASP chemistry utilises a two-step touchdown PCR method, with the elongation and annealing steps incorporated into a single step. The temperature used for the annealing stage determines the specificity of the reaction and hence the ability of the primers to anneal to the DNA template. A touchdown PCR involves starting with a high annealing temperature and incrementally decreasing the annealing temperature each PCR cycle. The higher annealing temperatures in the early cycles of a touchdown ensure that only very specific base pairing will occur between the DNA and the primer, hence the first sequence to be amplified is most likely to be the sequence of interest. The annealing temperature is gradually decreased to increase the efficiency of the reaction. The regions that were originally amplified during the highly specific early touchdown cycles will be further amplified and outcompete any non-specific amplification that may occur at the lower temperatures.

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KASP thermal cycling conditions

Recycling protocol

If clear genotyping clusters have not been obtained after completion of the standard KASP thermal cycle, the reaction plate should be thermally cycled further. One KASP recycling step comprises of three additional PCR cycles, as outlined in the table below.

Protocol stage	Temperature	Duration	Number of cycles for each stage
Stage 1 Amplification	94 °C	20 seconds	x 3 cycles
	57 °C	60 seconds	
Optional stage 2 (read stage for qPCR instruments only)	30 °C (any temperature below 40 °C is suitable for the read stage)	60 seconds	x 1 cycle

* Please note that stage 2 of the above program is only required if running and reading KASP genotyping reactions on a qPCR machine. If running the KASP thermal cycle program on a Peltier block or a Hydrocycler, only Stage 1 is needed although you must ensure that the reaction plates are cooled to <40 °C before performing the template read.

For a detailed explanation of recycling and an illustration of the effect that this can have on your genotyping results, please view our guide to further cycling of genotyping reactions.

For any queries about running KASP reactions in your laboratory please contact the technical support team at techsupport@lgcgroup.com.

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