KASP PCR Mix

For research use only

Components

Component	P4021	P4022
2X KASP PCR Mix	1 ml	1 ml × 5
Nuclease-free Water	1 ml	1 ml × 5

Storage

Store at -20°C, avoid repeated freezing and thawing.

Description

KASP PCR Mix is a 2X PCR Mix solution developed for KASP, containing double-antibody modified hotstart Taq DNA polymerase, dNTPs, buffer and other essential components for PCR amplification (except templates, primers and probes). When used, only templates, primers and probes need to be added into the amplification system, greatly simplifying the operation process, shortening the operation time, and reducing pollution (less sampling times). The reaction system of KASP PCR Mix is specially optimized to reduce the formation of amplification products by mismatched primers and significantly improve the specificity of PCR amplification.

KASP (Kompetitive Allele-Specific PCR) is a technique for genotyping based on allele-specific PCR. This technology utilises a unique form of competitive allele-specific polymerase chain reaction (PCR) that enables bi-allelic scoring of single nucleotide polymorphisms (SNPs) and insertions/deletions (Indels) at specific loci. KASP has the advantages of high throughput, low cost, and strong operability, and has been widely applied in fields such as genetic and improvement research of crop traits.

Applications

• SNP detection
• InDels detection

Features

• Convenient: pre-mixed, saving time and effort, and reducing the possibility of contamination
• High specificity: hotstart Taq DNA Polymerase
• High stability: the performance is not easy to change
• High sensitivity: fast, accurate, and high-throughput genotyping

Protocol

1.Preparation work before start

1.1Prepare DNA sample plate(s). For each genotyping assay to be run, include a minimum of 22 DNA samples to enable cluster analysis.

1.2No template controls (NTCs) should be included for every genotyping assay (2 NTCs on 96-well plates and 4 NTCs on 384-well plates).

1.3Thaw and vortex the required number of aliquots of KASP PCR Mix and KASP Assay Mix.

2.Preparation of reaction system

2.1 Prepare a sufficient volume of KASP genotyping mix (KASP Assay Mix + KASP PCR Mix) for each of the assays to be run, including a 10% excess to allow for pipetting. Please refer to the following table for the preparation of the reaction system on ice:

Reagent	96-well plate	384-well plate
template DNA [1]	5 μL	2.5 μL
2X KASP PCR Mix	5 μL	2.5 μL
KASP Assay Mix	0.14 μL	0.07 μL
Nuclease-free Water [2]	n/a	n/a
Total volume	10 μL	5 μL

Note:

[1] The recommended minimum final DNA concentration in KASP genotyping reactions is 2.5 ng/μL (based on human genome size).

[2] Nuclease-free Water can be ordered separately (Cat. No.: P9022 / P9023).

2.2 Dispense the required volume of prepared KASP genotyping mix into each well of the DNA plate.

2.3 Seal the prepared reaction plate with a PCR-suitable, optically clear seal.

2.4 Centrifuge the plate briefly to ensure all reaction volume is at the bottom of each well.

3. Run the KASP thermal cycle

Load the reaction plate into the thermal cycler or qPCR instrument. Refer to the following table to run the KASP reaction:

Stage	Temperature	Time	Number of Cycles
Hot-start Taq activation	95°C	1 min	1
Touchdown	95°C	15 sec	10
	56°C→50°C, – 0.6°C/cycle	15 sec	
	72°C	20 sec	
Amplification	95°C	15 sec	26
	50°C	15 sec	
	72°C	20 sec	
(optional) Read (qPCR instruments only)	30°C	60 sec	1

Note:

This product requires a lower annealing temperature and three-step amplification, otherwise the amplification efficiency is poor.

4. Analyze the results

4.1 Before performing a plate read, ensure that the plate is cooled to below 40°C. If the plate is not

read below 40°C, it will not be possible to analyse the genotyping data.

4.2 Perform the end-point plate read using a FRET-capable plate reader or qPCR machine. The following Table details the fluorophores for KASP:

Fluorophore	Excitation (nm)	Emission (nm)
FAM	485	520
HEX	535	556
ROX	575	610

4.3 Perform further PCR cycling of the reaction plate if necessary. The KASP recycle program is detailed in the following table:

Stage	Temperature	Time	Number of Cycles
Denaturation	94°C	20 sec	3
Annealing/Elongation	57°C	60 sec	

4.4 Store completed reaction plates in a dark fridge (~4°C for a maximum of 1 week) until data has been analysed. This will allow you to perform additional read(s) or recycle steps if required, to ensure you have obtained the best possible data.

4.5 Analyse raw data using cluster plots to enable genotypes to be assigned to the DNA samples.

Notes

1, Storage conditions: the product should be stored at -20°C. KASP PCR Mix should be aliquoted upon receipt to minimise the need for repeated freeze-thaw cycles.
2, No template controls (NTCs) should be included for every genotyping assay (2 NTCs on 96-well plates and 4 NTCs on 384-well plates).

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucl- eases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Product Use Limitations

This product is sold exclusively for research purposes and in vitro use. Neither the product, nor any individual components, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, available upon request.