

Super KP PCR Master MixPlus (Low ROX+)

Cat. No./Spec.

Cat. No.	P4051	P40452
Spec.	1 ml	60 ml

Components

Component	P4051	P4052
2X Super KP PCR Master MixPlus (Low ROX+) *	1 ml	60 ml

* It contains Hotstart Taq DNA polymerase, dNTPs/dUTP, ROX Reference Dye, Heat-labile UDG, FAM, HEX-labeled probes, and their quencher probes.

Storage

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

Description

Super KP PCR Master MixPlus (Low ROX+) is a single-tube premix developed for competitive allele-specific PCR, incorporating a UDG/dUTP system to prevent carryover contamination. It is pre-supplied with FAM- and HEX-labeled probes, allowing the reaction to be set up simply by adding the SNP-specific primers and DNA template. The reaction system has been specially optimized to minimize mispriming- derived products and significantly enhance PCR specificity, enabling accurate detection of single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) at target loci. With its advantages of high throughput, low cost, and excellent operational convenience, this product has broad applications in crop trait genetics and improvement research, animal breeding, and medical studies.

This product contains a low concentration of ROX reference dye, making it compatible with real- time PCR instruments that require ROX normalization, such as the ABI Q3 series. It is also suitable for instruments that do not require ROX calibration or that have the ROX channel switched off.

Applications

- SNP detection
- InDels detection

Features

- Including FAM and HEX labeled probes
- 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

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

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

1.3 Thaw and vortex the required number of aliquots of 2X Super KP PCR Master MixPlus (Low ROX+) and Primers Mix (Self-provided).

2.Preparation of reaction system

2.1 Prepare a sufficient volume of genotyping 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	Volume (per reaction)	
	96-well plate	384-well plate
template DNA ^[1]	5 µL	2.5 µL
2X Super KP PCR Master MixPlus (Low ROX+)	5 µL	2.5 µL
Primers Mix	0.14 µL	0.07 µL
Nuclease-free Water	n/a	n/a
Total volume	10 µL	5 µL

Note:

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

2.2 Dispense the required volume of prepared 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 PCR thermal cycle

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

Stage	Temperature	Time	Number of Cycles
Hot-start Taq activation	95°C	60 sec	1
Touchdown	95°C	15 sec	10
	61°C→55°C, – 0.6°C/cycle	15 sec	
	72°C	20 sec	
Amplification	95°C	15 sec	26
	54°C	15 sec	
	72°C	20 sec	
(optional) Read (qPCR instruments only)	30°C (Below 40°C)	60 sec	1

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.

Fluorophore information is as follows:

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 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. PCR Master 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, exodeoxyribonucleases 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.