Vazyme

Vazyme biotech co., ltd.

One Step U+ Probe Mouse Genotyping Kit

PD104-01

Version 8.1

Introduction

One Step U+ Probe Mouse Genotyping Kit is designed for rapid genotyping of mouse genotypes. The kit comprises a whole set of animal tissue DNA extraction and anti-contamination probe method QPCR amplification system, which is can be used to rapidly release genomic DNA from tissues such as mouse tail, ear and toe. The product is directly subjected to QPCR amplification, avoiding multiple operations such as capping and pipetting. The genotype results are directly analyzed by software to avoid manual checkup that can greatly reduce the time spent on the experiment. The dUTP/UDG anti-contamination system in the reagents rapidly degrades U-containing contaminants at room temperature, significantly reducing aerosol contamination and improving detection throughput and reproducibility of results.

Package Information

Components	PD104-01 (200 rxn)
1 x Mouse tissue Lysis Buffer	2 x 20 ml
Proteinase K	800 µl
2 × AceQ U+ Probe Master Mix[a]	2 x 1.25 ml
50 × ROX Reference Dye 1[b]	100 µl
50 × ROX Reference Dye 2[b]	100 µl

a. Contains AceTaq®DNA Polymerase,dNTP Mix,Mg^{2+}.

b. Used to correct the fluorescence signal error generated between the hole and the hole. 50 x ROX Reference Dye 1 for ABI 7900HT/7300 Real-Time PCR System and StepOnePlus®; ABI 7500, 7500 Fast Real-Time PCR System, Stratagene Mx3000P for 50 × ROX Reference Dye 2; Roche, Bio-Rad Real Time PCR machine does not have to use ROX

Storage Conditions

Store 1 x Mouse tissue Lysis Buffer at 4˚C; the rest of the components are stored at -20˚C; try to avoid repeated freezing and thawing during use. If the amount of use is small, it is recommended to aliquot into small parts.

Application Range

Mouse genotyping

Mouse transgenic detection

Mouse gene knockout analysis

Experimental operation and precautions

1. Please mix the Mix upside down before use. Do not vortex to avoid excessive bubbles, which will cause the inaccuracy volume of the reaction system, thus affecting the quantitative results. The Mix can be used after short centrifugation. It should be lightly blown during use. If the Mix is foamed, it needs to be centrifuged again before use.
2. This product is based on AceTaq® DNA Polymerase which is a chemically modified hot start enzyme. The pre-denaturation temperature should be set at 95˚C for at least 5 minutes to fully release the enzyme activity. If the GC content of the template is high, the pre-denaturation time can be extended to 10 minutes.
3. The pollution digestion step of the product can effectively remove the interference of the urea-containing PCR product aerosol on the system, and the step can be omitted without affecting the subsequent PCR reaction.

Application Example

1. DNA extraction

Recommended tissue usage: 0.5 - 1mm mouse tail tip / 1 - 3mm 2 mouse ear / 0.5 mouse toe

1/ Prepare 1x lysate according to the number of samples to be lysed, properly enlarge the number. The preparation method of the lysate required for a single sample is as shown in the table below:

1x lysate (a single sample)	
Proteinase K	4 μl
1 x Mouse tissue Lysis Buffer	200 μl

1/ In a 1.5 ml of sterile EP tube, add 200 μl of 1x lysate to the desired lysed tissue, vortex and incubate for 10 - 20 min in a 55˚C water bath. To ensure DNA release efficiency, be sure to completely immerse the tissue into the lysate. For the recommended tissue usage, a 20 min incubation is sufficient to release a sufficient amount of DNA template.

2/ After the incubation is complete, place the sample in a 95˚C or boiling water bath for 5 min to inactivate Proteinase K.

3/ Mix the lysate thoroughly by vortexing, centrifuge at 12000 rpm for 5 min. The supernatant can be used for QPCR reaction, and it can also be transferred to another sterile EP tube for -20˚C for at least three months.

2. QPCR Reaction

The test equipment model is QuantStudioTM Real-Time PCR 6.

1/ Preparation of qPCR reaction system

2 x Genotyping Probe Master Mix	10 μl
Primer 1F (10 μM)	0.4 μl
Primer 1R (10 μM)	0.4 μl
Primer 2F (10 μM)	0.4 μl
Primer 2R (10 μM)	0.4 μl
Taqman Probe 1 (10 μM)	0.2 μl
Taqman Probe 2 (10 μM)	0.2 μl
ROX Reference Dye 2	0.4 μl
Tempalte DNA	X μl
sterilized distilled water	Up to 20 μl

Note: The amount of each component in the reaction system can be fine-tuned according to the following principles:

a. The final primer concentration can be adjusted in the range of 0.1 - 1.0 μM.

b. The final probe concentration can be adjusted between 50 nM and 250 nM.

c. Please select the length of the amplified product in the range of 80 - 200 bp.

d. The volume of the template containing the salt ions should not exceed 1/10 of the reaction volume.

2/ Perform qPCR reaction under the following conditions:

Pollution digestion	Reps:1	37˚C	2 min
Pre-denaturation	Reps:1	95˚C	5 - 10 min
Cycle reaction	Reps:40 - 45	95˚C	10 sec
		60˚C	30 sec

Note:

a. AceTaq® DNA Polymerase requires heat activation to restore enzyme activity. Please set the PCR reaction pre-denaturation condition to 95˚C for 5 minutes. If the GC content of the template is high, the pre-denaturation time can be extended to 10 minutes.

b. Extension time Please adjust according to the minimum time limit of data collection required by your QPCR instrument: at least 30 seconds when using ABI 7700 and 7900HT; at least 31 seconds when using ABI 7000 and 7300; at least 34 seconds when using ABI 7500.

c. After the end of the reaction, the results were analyzed to confirm the QPCR allele detection scatter plot. The genotype of the corresponding well can be directly exported by export for pasting records.

FAQ and Solutions

1.Frequently asked questions about probe method QPCR (Please see Vazyme product Q113 instructions).

2.Problems with dual probe genotype identification experiments (software analysis related issues are based on Q6 Realtime PCR Software)

◇The effect of excessive digestion and excessive digestion time on the system

Please refer to the recommended tissue dosage and digestion time for tissue digestion. A recommended size tissue digested for 20 minutes generally yields a DNA concentration of 1 - 2 ng/μl. Excessive digestion and excessive digestion time will cause a large increase in the impurity components in the system, affecting the subsequent QPCR reaction. The recommended size of the mouse tissue digested for 10 - 20 minutes can generally obtain better amplification results. Digested solution with a mouse tissue weight of more than 0.01 g and digested for more than 6 hours will no longer be suitable as a template for subsequent QPCR reaction detection.

◇ The effect of too small digestive tissue and too short digestion time on the system

If the digestive tissue is too small (less than 0.0005 g), or the normal size of the tissue is digested for too short (less than 5 minutes), it may not be able to release enough DNA as a template for subsequent amplification, and increase the template volume in the PCR system in order to increase the amount of template will bring into the unsuitable salt ions, it is not recommended for rapid probe method QPCR reaction.

◇The function of the template-free sample

Whether to set the template-free sample will not affect the software's judgment on the result analysis. It is recommended to set a template-free sample for each experiment under the condition that the experimental system is not fully mature, which can effectively judge potential problems such as system pollution and poor amplification specificity. In the mature detection system, the setting of the template-free sample can be considered.

◇ Unrecognized due to concentration difference

In the same quantitative typing experiment, if the DNA concentration difference of different samples is too large (for example, there are more than 3 or more differences in Ct values at individual points), this point may not be correctly judged by the system. Therefore, the concentration of a DNA sample used for testing should be as uniform as possible, that is, the content and size of original tissue sample should be consistent, and the digestion time is consistent, so that an uniform genomic concentration can be obtained. If it is necessary to have a template with a large difference in concentration in one reaction, the template should be diluted and adjusted according to the expected concentration difference in advance to achieve the best analysis result.

◇ Unrecognized by the difference in amplification efficiency between two sets of primers

Two sets of primers customized for the two genotypes may have potential interactions. The difference in amplification efficiency may lead to software misjudgment of heterozygous genotypes. This factor should be considered when designing primers and the amplification efficiency of the single primer/probe system should be determined before the experiment, in order to make the two primer/probe systems have close amplification efficiency, and the amplification curves should be as close as possible for heterozygotes.

◇ Experiments for identifying different genes in a single reaction need to be analyzed separately

Experiments with different primer/probe systems for different target genes can be completed in one on-board reaction, and the results do not interfere with each other. However, it is necessary to pay attention to distinguishing the template sample well area of different target genes when adding, and choose the correct wells of all the same target genes when analyze the results.

◇ A set of experiments does not completely include wild-type, mutant homozygous and mutational heterozygous results in incorrect analysis results

A complete set of genotypes that do not completely include wild-type, mutant homozygous, and mutant heterozygous in a set of experiments can result in software that cannot judge (all unidentified) or misjudge the genotype of the sample (eg, the hybrid is judged to be wild in the absence of a wild-type control), so all three genotype samples must be included in one experiment. If it is not expected to be included, a control well for determining the genotype should be additionally set to ensure the correct judgment of the unknown sample by the software.