Mouse Tissue Direct PCR Kit

Fastest DNA Extraction from Mouse Tissue Followed by PCR

www.tiangen.com/en

Mouse Tissue Direct PCR Kit

Cat. No. GKG205

Product Contents

Contents	GKG205-01 (25 µl × 50 rxn)	GKG205-02 (25 µl × 200 rxn)
Tissue Lysis Buffer	5 ml	20 ml
Digestive Enzyme	200 µl	800 µl
2× Dir PCR MasterMix	625 µl	2 × 1.25 ml
RNase-Free ddH_2O	1 ml	2 × 1 ml
Handbook	1	1

Storage

Tissue Lysis Buffer and Digestive Enzyme can be stored for 15 months at room temperature (15-30°C); 2× Dir PCR MasterMix can be stored for up to 15 months at -30~-15°C, and repeated freeze-thawing will not affect its activity.

Introduction

The kit adopts a unique packaging system, which contains all reagents for rapidly extraction of mouse tissue genome DNA and subsequent PCR amplification. It is suitable for extracting genome DNA from mouse tail, ears, toes and other tissues in one step and for subsequent PCR amplification and detection. The whole extraction process does not include homogenization, smashing, overnight digestion, phenol/chloroform extraction, DNA precipitation or column purification, etc. Stable and reliable results can be achieved with simple and fast experimental operation.

The 2× Dir PCR MasterMix provided by the kit is a PCR reagent with high amplification compatibility and can perform high-efficiency and specific amplification without completely removing impurities such as proteins. The premixed MasterMix comprises antibody modified *Taq* DNA polymerase, dNTPs, $MgCl_2$, reaction buffer, PCR reaction enhancer and stabilizer. Only a crude extraction template and primers are needed for subsequent PCR detection. The premixed MasterMix has the characteristics of simple operation, high sensitivity, strong specificity, good stability, etc., and is especially suitable for high-throughput detection and screening. The MasterMix contains electrophoretic dye, which can be used for electrophoresis detection directly after the reaction. The PCR product has a dA-tailing, which can be used for TA cloning.

Features

Easy and fast: Suitable for one-step gene identification from mouse tail, ears, toes and other tissues.

High specificity: The *Taq* polymerase used in this product is antibody modified HotStart polymerase, with high amplification specificity and high affinity for template and primers, and is especially suitable for genotyping and transgene identification.

Gene detection: This product is easy to operate and produces reliable results, which is especially suitable for high-throughput analysis and detection of mice.

Important Notes Before Starting

1. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the extraction yield will be decreased.
2. The Tissue Lysis Buffer should be stored at room temperature. If a precipitate has formed in Buffer, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate.
3. The 2× Dir PCR MasterMix provided by this product is 2× Mix. Template, primers and sterilized water should be added to make up to a 1× solution before use.

Protocol

1. Before using this kit, please carefully check whether there is precipitation in Tissue Lysis Buffer. If precipitation forms, please fully balance the buffer at room temperature until the precipitation is completely dissolved, or redissolve the precipitate in 37°C water bath and shake well before use. Dissolve the Tissue Lysis Buffer and store at room temperature.
2. Prepare digestion buffer according to the following formula:

Component	volume
Tissue Lysis Buffer	96 µl
Digestive Enzyme	4 µl
Total	100 µl

Note: Please prepare the digestion buffer right before use to ensure the activity of Digestive Enzyme.

3. Take a small amount of mouse tissue samples (about 5~10 mg) into a 1.5 ml centrifuge tube and add 100 µl of digestion buffer. Ensure that the tissue samples are completely soaked in the digestion buffer and incubate at 65°C for 30 min. During this period, flick the tube bottom every 10 min to improve digestion efficiency.
4. After digestion is completed, instantaneously centrifuge the mixture and process at 95 ~ 100°C for 5 min.
5. Take 1 µl supernatant for PCR reaction. Please refer to the PCR system and amplification procedure below:

Reference Reaction System

Set up a 25 μl PCR reaction system as follows:

Component	volume
2× Dir PCR MasterMix	12.5 μl
Forward primer (10 μM)	0.5 μl
Reverse primer (10 μM)	0.5 μl
Template DNA	1.0 μl
RNase-Free ddH_2O	Add to 25 μl

After all the reagents have been added, mix well and centrifuge instantaneously to collect all the reagents to the bottom of the tube.

Reference Reaction Program

Temperature	Time	Cycles
95°C	3 min	1 cycle
94°C	30 sec	35 cycles
55°C △1	30 sec	
72°C	1 kb/min	
72°C	5 min	1 cycle
4°C	Holding	1 cycle

△1 In general, the annealing temperature of the primer is 5°C lower than the melting temperature (Tm), and the specific annealing temperature setting can be adjusted according to different primers.

PCR Product Detection

After the reaction is completed, take 5-10 μl of the reaction product for agarose gel electrophoresis detection.

Note: Examples are provided for reference only. The actual reaction conditions vary according to the structure of templates, primers, etc. The best reaction conditions should be set according to the actual situation. If liquid is found on the tube wall or cover during operation, centrifuge instantaneously to the bottom of the tube.