EZ-press Tissue Direct PCR Kit

Catalog No.: EZB-TDP1

Description

The ***EZ*Bioscience**® **EZ-press Tissue Direct PCR Kit** is specially used for mouse genotyping, including DNA releasing and PCR amplification system. This kit can quickly release complete genomic DNA from mouse ears, tails, toes, and other tissues without the need for homogenization, overnight digestion, phenol-chloroform extraction, DNA precipitation, or column purification, which greatly shortens the experimental operation time. During use, directly immerse the tissue into Lysis Buffer premixed with Proteinase Mix, incubate at 55°C for 15 minutes to lysis tissue, and then heat at 95°C for 5 minutes to inactivate Proteinase. After centrifugation, the supernatant of the lysate can be directly used as a template for PCR amplification.

The 2x Direct-PCR Mix (Dye plus) in this kit contains Taq DNA Polymerase, dNTPs and a highly optimized buffer system with superior amplification and anti-interference ability. In the PCR reaction, only the template and primers need to be added to carry out the amplification, which reduces operations such as pipe opening and pipetting, and significantly improves the detection throughput and reproducibility of results. The dye is also premixed in the mix, and loaded directly after the PCR reaction. The PCR product has a base A at the 3' end and can be directly cloned into a T vector. This kit has a wide range of applications, and is especially suitable for mouse genotyping, transgenic detection and gene knockout analysis.

Components

Components	EZB-TDP1 (200 Rxns)	EZB-TDP1-L (500 Rxns)
Lysis Buffer	20 ml	50 ml
Proteinase Mix	0.4 ml	1 ml
2x Direct-PCR Mix（Dye plus）	2 ml	5 ml

Storage

Store the Lysis Buffer at 2 ~ 8°C and protected from light. Store other components of this product at -20°C.

Application

Apply to mouse genotyping, mouse transgene detection, and mouse gene knockout analysis.

Notice

1. The step of Proteinase inactivation (95°C for 5 min) must be performed, otherwise its residual activity will inhibit the subsequent PCR reactions.

2. The preparation of PCR reaction system should be performed on ice to improve the specificity of amplification.

Protocol

DNA Extraction

1. The suggested tissue weight according to tissue types:

Tissue types	mouse ear	mouse tail	mouse toe
Volume of use	2 ~ 5 mm^2	1 ~ 3 mm	1 ~ 2

2. Prepare Tissue Digestion Solution according to the number of tissue samples that need to be lysed. The method for preparing Tissue Digestion Solution for single sample is as follows:

Components	Tissue Digestion Solution (single sample)
Proteinase Mix	2 μl
Lysis Buffer	100 μl

The Tissue Digestion Solution should be prepared and used immediately. After adding each reagent, use a pipette or vortex to mix it before use.

3. Add 100 ul of freshly prepared Tissue Digestion Solution to the tissue sample. After vortexing, incubate at 55°C for 15 minutes. During lysis, make sure that the tissue is completely immersed into the Tissue Digestion Solution.

For target fragments with regular size, incubation for 15 minutes is sufficient to release sufficient DNA template. The incubation time can also be adjusted according to the actual situation. The following table shows the recommended incubation time for amplified fragments of different lengths at 55°C:

Amplified fragment length	Recommended incubation time at 55°C
~ 500 bp	10 min
~ 1000 bp	20 min
~ 1500 bp	30 min

Note: After incubation, the tissue may still look intact, but a sufficient amount of genomic DNA has been released, which is sufficient for PCR detection.

4. After that, place the tissue lysates at a 95°C, and incubate for 5 minutes to inactivate the proteinase activity.

5. After the lysate is vortexed and mixed thoroughly, centrifuge at 12,000 rpm for 5 minutes. The supernatant can be used as the template for the PCR reaction. The digested supernatant can be stored at -20°C for 3 months.

PCR Amplification

1. Before use, take the 2x Direct-PCR Mix (Dye plus) from the refrigerator at -20°C, and place it at room temperature for 5 ~ 10 minutes or hold the reagent tube by hand to fully melt it, then centrifuge briefly to the bottom of the tube and place on ice for use. **Preparation of the reaction system on ice:**

Components	Volume/20 μl	Volume/50 μl
Supernatant of the digestion product*1	1 μl	2 μl
2x Direct-PCR Mix (Dye plus)	10 μl	25 μl
Forword Primer(10 μM)	0.5 μl	1 μl
Reverse Primer(10 μM)	0.5 μl	1 μl
ddH_2O	to 20 μl	to 50 μl

***1:** The amount of the supernatant of the digestion product should not exceed 1/10 of the total volume of the PCR reaction.

After the PCR reaction system is prepared, Pipet up and down for 10 ~ 20 times to **mix thoroughly (important)** and briefly to the bottom of the tube, then perform PCR reaction.

2. The PCR reaction program can be set up as follows:

Step	1	2			3
	Pre-denature	PCR			Extend completely
Cycle	1	35			1
		Denature	Anneal*	Extend	
Temp.	94°C	94°C	55 ~ 60°C	72°C	72°C
Time	5 min	30 sec	30 sec	30 sec/ Kb	5 min

***:** The annealing temperature needs to be adjusted according to the Tm value of the primer. Generally, the annealing temperature should be set to 1 ~ 2°C lower than the Tm value of the primer.

3. The amplification products can be directly detected by agarose gel electrophoresis without need to add DNA Loading Buffer.

Trouble Shooting

Problems	Possible Cause(s)	Suggestion(s)
The amount of amplified product is low or cannot be amplified	The annealing temperature is set too high	Reduce the annealing temperature by 3°C each time
	The PCR cycles is not enough	Increase the number of cycles appropriately
	PCR primers have quality problems	Redesign primers
	The amount of template is too low	Properly increase the amount of template
	Tissue digestion is inadequate	Extend incubation time at 55°C to 30 min
	Protease is not completely inactivated	Inactivation step can be performed in a boiled water bath
Non-specific amplification	The annealing temperature is too low	Increase the annealing temperature
	PCR primers are mismatch	Redesign primers
	When the PCR reaction system is prepared, the temperature is too high or the preparation time is too long after the preparation is completed	The preparation of the PCR reaction system is carried out on ice, and the PCR amplification reaction is carried out as soon as possible after the preparation is completed