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Plant Tissue Direct PCR Kit (With Dye)

Product description

Plant Tissue Direct PCR Kit (With Dye) is a kit that can directly perform PCR amplification on leaves of different types of plants. It has wide adaptability and strong stability. The kit uses a unique lysis buffer system, which can quickly lyse a variety of plant samples and release genomic DNA. It does not need to remove proteins, RNA or secondary metabolites, etc., and the released genomic DNA can be used directly as a template for PCR reaction. In addition, the sample usage is small, and experiments can be performed with as little as 1 mm plant leaves. The 2× Plant Master Mix provided in this kit has strong amplification compatibility and can directly use the lysate of the sample to be tested as a template for efficient and specific amplification. This reagent is a 2-fold concentrated PCR reaction mixture, which contains all components used for PCR amplification except templates and primers, greatly simplifying the operation process and reducing the chance of contamination. It also contains a tracer dye, and the PCR product can be directly electrophoresed. This kit can be used for transgenic plant identification, plant genotyping, etc.

Specifications

Cat.No.	10187ES05 / 10187ES50 / 10187ES70
Size	5 T / 50 T / 200 T

Components

Components No.	Name	10187ES05	10187ES50	10187ES70
10187-A	Buffer P1	250 μL	1.25 mL × 2	5 mL × 2
10187-B	Buffer P2	50 μL	500 μL	1 mL × 2
10187-C	2 × Plant Master Mix*	50 μL	500 μL	1 mL × 2

【Note】： *2× Plant Master Mix: Contains hot-start Taq DNA polymerase, dNTP mixture, $MgCl_2$, reaction buffer, PCR reaction enhancer, optimizer, stabilizer, etc. It also contains electrophoresis loading buffer, which can be used for direct electrophoresis after PCR is completed.

Storage

1. Reagent 10187-A [Buffer P1], store at 2-8°C. Valid for 12 months.
2. Reagent 10187-B [Buffer P2], neutralizes the lysis product, which is conducive to long-term storage of samples, and is stored at 2-8°C. Valid for 12 months.
3. Reagent 10187-C [2 × Plant Master Mix], store at -25~-15°C, avoid repeated freezing and thawing. Valid for 12 months.

Instructions

1. Plant leaves

1) Grinding and lysis method:

a. Grinding: Place leaves with a diameter of about 5 mm in 50 μL Buffer P1, and use a grinder with steel balls (steel balls with a diameter of about 3 mm, 2 in total) to crush the leaves (45 Hz, 1 min). After the leaves are crushed, the solution turns green. Centrifuge instantly. Keep the supernatant at 4°C for later use. Take 1 μL for PCR amplification.

b. Pistol tip crushing: It is recommended to use young leaves. Place leaves with a diameter of about 5 mm in 50 μL Buffer P1, and crush the leaves with a gun tip. After crushing, the solution turns green. Centrifuge instantly. Keep the supernatant at 4°C for later use. Take 1 μL for PCR amplification.

2) Heating lysis method: It is recommended to use young leaves. Place a leaf with a diameter of about 5 mm in 50 μL Buffer P1 and heat at 95°C for 5-10 min (make sure the lysis solution completely submerges the leaf). Leaves that are difficult to lyse (old leaves) can be appropriately extended (10-20 min). The solution turns green after heating and lysis. Shake and mix, centrifuge instantly, and keep the supernatant at 4°C for later use. Take 1 μL for PCR amplification.

3) Direct method: It is recommended to use young leaves. Use a hole puncher or knife to directly add leaves with a diameter of about 1 mm to the PCR reaction system; for complex samples or long fragment amplification, it is recommended to use leaves with a diameter of <1 mm.

2. PCR reaction system

Table 1 Reaction system

Components	Volume (μL)	Volume (μL)	Final concentration
2× Plant Master Mix	10	25	1×
Forward Primer (10 μM)	0.5	1	0.2-0.25 μM
Reverse Primer (10 μM)	0.5	1	0.2-0.25 μM
Cleavage product (DNA template)	1	2	-
ddH_2O	To 20	To 20	-

【Note】：*Each component should be fully mixed before use.

1) Template addition amount: less than 5% of the PCR reaction system. Too much will seriously inhibit the PCR reaction. It is strongly recommended to add 1 μL template. The grinding and lysis method is preferred for direct leaf amplification.

2) Primer final concentration: 0.2-0.25 μM can obtain better results. When the reaction performance is poor, the primer concentration can be adjusted within the range of 0.1-0.5 μM.

3) Reaction system: It is recommended to use 20 μL or 50 μL to ensure the effectiveness and repeatability of target gene amplification.

4) System preparation: Prepare the PCR reaction system, place it on a vortexer to vortex and mix it, and centrifuge it instantly to collect the reaction solution at the bottom of the tube.

5) Control reaction: It is recommended to set up positive and negative PCR control reactions when performing PCR to eliminate the interference of false positives or false negatives.

6) In order to preserve the lysed template more stably, the transferred supernatant is mixed in a ratio of lysate (DNA template): Buffer P2 = 5:1, and stored at -20°C after mixing. Stable storage varies with time and sample status. If the treated plant leaf supernatant is used for PCR amplification within one week, Buffer P2 is not needed and the supernatant should be stored at -20°C.

3. Reaction conditions

Table 2 Reaction conditions

Cycle steps	Temperature (°C)	time	Number of cycles
Pre-denaturation	94	5 min	1
denaturation	94	10 sec	35
annealing*	50-65	20 sec	
extend**	72	1 min/kb	
Final extension	72	5 min	1

【Note】：*Annealing temperature: Please refer to the theoretical Tm value of the primer. The annealing temperature can be set 2-5°C lower than the theoretical value of the primer.

**Extension time: It needs to be determined according to the length of the fragment. For DNA fragments within 1 kb, the recommended extension time is 1 min.

Notes

1. When doing leaf experiments, it is recommended to use freshly collected leaf tissue. If it is long-term frozen tissue, it needs to be stored at -80°C. Repeated freezing and thawing should be avoided as much as possible to avoid template degradation and affect PCR efficiency. Young and tender leaf tissue is preferred. If it is a mature leaf, avoid using the main vein tissue of the leaf.
2. It is recommended that the length of the amplified fragment be within 1 kb to optimize the amplification efficiency.
3. When sampling, use a puncher or knife to take samples of appropriate size. If the samples are different, the puncher or knife needs to be cleaned before each sample processing.
4. For leaf tissue, it is recommended to take 1-10 mm leaves. Too small will result in low PCR amplification yield, and too much will inhibit the PCR reaction. Use the heating lysis method, gun tip crushing, and grinder crushing to process plant leaves. After processing, it needs to be shaken and centrifuged. Be sure to take the supernatant for testing. Precipitation will seriously inhibit the PCR reaction.
5. For your safety and health, please wear a lab coat and disposable gloves when operating.
6. This product is for scientific research purposes only!

Common Problems and Solutions

Frequently asked questions	Possible causes	Workaround
There were no bands in the positive control and the samples to be tested.	The PCR reaction system or reaction conditions are not suitable.	Use gradient PCR to explore the optimal PCR reaction conditions.
	PCR reagents lose activity if not stored properly.	2×PCRMix should be stored at -25~-15°C and avoid repeated freezing and thawing during use. If used frequently, it can be stored at 2-8°C for a short period of time.
	Primer design issues.	Try redesigning the primers to check.
The positive control has the target band, while	Improper storage or long-term storage may cause loss of reagent activity.	Use fresh reagents.

the sample to be tested has no band or the band is weak.	The proportion of lysis solution and neutralization solution added is inappropriate, and the lysis mixture affects the pH value of the PCR system.	Under normal conditions, the pH of the neutralized lysis mixture should be around 7-8 (the lysis product and Buffer P2 are strictly neutralized in a 5:1 ratio).
	The sample lysis mixture was improperly stored or stored for too long, and the DNA genome has been degraded.	The lysis mixture can be stored at 2-8°C for 5 days. Try to use freshly prepared lysis mixture for PCR.
	The amount of template added is not appropriate.	Optimize the template addition amount within the reaction system range of <5%.
	Insufficient number of PCR cycles.	Increase the number of PCR cycles appropriately, and 35-40 cycles are recommended. Because the template is complex, the PCR reaction should generally be 5-10 cycles more than when using purified DNA templates.
Non-specific amplification	PCR annealing temperature is too low, cycle number, primer concentration, or template concentration is too high.	Increase the PCR annealing temperature, decrease the number of PCR cycles, primer concentration, or template concentration.
	PCR primer mismatch.	Redesign PCR primers.
	The temperature was too high when preparing the PCR reaction system or the system was left for too long after preparation.	The PCR reaction system is prepared at low temperature, and the PCR amplification reaction is carried out as soon as possible after the preparation is completed.
Negative control shows target band	Contamination of operating tools or reagents.	All reagents and equipment used in the experiment should be sterilized by high pressure. Be careful and gentle when operating to prevent the target sequence from being sucked into the sample gun or splashing out of the centrifuge tube.
	Cross contamination between samples.	Each sampler is used for only one sample; or after taking a sample, immerse the cutting edge of the sampler in 2% sodium hypochlorite solution, rinse it repeatedly, and then wipe off the residual liquid with a clean paper towel.