Plant Tissue Direct PCR Kit (With Dye)

Product Information

Product name	Cat#	Size
Plant Tissue Direct PCR Kit (With Dye)	10187ES50	50 T
	10187ES70	200 T

Product Description

Plant tissue direct PCR kit is a kit that can directly amplify different types of plant leaves by PCR, with 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. The released genomic DNA can be used directly as a template without removing protein, RNA, or secondary metabolites in PCR reaction. In addition, the kit requires a small amount of sample, as low as 1 mm plant leaves can be used for experiments.

The 2×Plant Master Mix provided in this kit has strong amplification compatibility and can directly use the lysate of the sample as a template for efficient and specific amplification. This reagent is a 2-fold concentrated PCR reaction mixture, which contains all the components used for PCR amplification except the template and primers, which greatly simplifies the operation process and reduces the chance of contamination.

The kit can be used for identification of transgenic plants, plant genotyping, etc.

Product Components

Component Number	Components	Cat#/Size	
		10187ES50 (50 T)	10187ES70 (200 T)
10187-A	Buffer P1	1.25 mL × 2	5 mL × 2
10187-B	Buffer P2	500 μL	1 mL × 2
10187-C	2 × Plant Master Mix[a]	500 μL	1 mL × 2

[Note]: a: **2× Plant Master Mix:** Contains hot-start Taq DNA polymerase, dNTP mix, $MgCl_2$, reaction buffer, PCR reaction enhancer, optimizer and stabilizer, etc. It also includes electrophoresis Loading Buffer, which can be directly used in electrophoresis after PCR.

Shipping

The products are shipped with ice packs.

Storage

1. Reagent 10187-A [Buffer P1] can be stored at 4℃ for 1 year.

2. Reagent 10187-B [Buffer P2], used for neutralizing lysate, which is beneficial to store the sample for a longer time, and can be stored at 4℃ for 1 year.

3. Reagent 10187-C [2× Plant Master Mix] can be stored at -20℃ for 1 year. Avoid repeated freezing and thawing.

Operate

Plant leaves

Grinding and cracking method:

①Crushing with a grinder: Put the leaves with a diameter of about 5 mm in 50 μL Buffer P1, add steel beads (about 3 mm in diameter, 2 in total) in grinder to break the leaves (45 Hz, 1 min), and the solution with broken leaves will appear green. Centrifuge briefly, keep the supernatant at 4°C for later use, and take 1 μL for PCR amplification.

②Mashing with pipette tip: It is recommended to use young leaves. Place leaves with a diameter of about 5 mm in 50 μL Buffer P1, mash the leaves with a pipette tip, the solution turns green after mashing. Centrifuge briefly, keep the supernatant at 4°C for later use, and take 1 μL for PCR amplification.

Thermal cracking method: It is recommended to use young leaves. Place leaves with a diameter of about 5 mm in 50 μL Buffer P1 and heat at 95°C for 5-10 min (to ensure that the lysate is completely submerged in the leaves), for leaves that are difficult to lyse (old leaves), the time can be appropriately extended (10-20 min). The solution turns green after heating and lysing. Shake and mix well, centrifuge briefly, keep the supernatant at 4°C for later use, and take 1 μL for PCR amplification.

Direct method: It is recommended to use young leaves. Use a hole punch or a knife to directly add leaves with a diameter of about 1 mm into the PCR reaction system; for complex samples or amplification of long fragments, it is recommended to use leaves with a diameter of less than 1 mm.

PCR Reaction System

Components	Volume (μL)	Volume (μL)	Final Concentration
2× Plant Master Mix	10	25	1×
Forward Primer (10 μmol/L)	0.5	1	0.2-0.25 μmol/L
Reverse Primer (10 μmol/L)	0.5	1	0.2-0.25 μmol/L
Cleavage product (DNA template)	1	2	-
ddH_2O	To 20	To 50	-

[Note]: All components should be thoroughly mixed before use.

a) The amount of template added: less than 5% of the PCR reaction system. Excessive amount will seriously inhibit the PCR reaction. It is strongly recommended to add 1 μL template. For direct amplification of leaves, the grinder cracking method is preferred.

b) Final primer concentration: Using 0.2-0.25 μmol/L primer to get better results. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1-0.5 μmol/L.

c) Reaction system: 20 μL or 50 μL is recommended to ensure the validity and repeatability of target gene amplification.

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

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

f) In order to store the lysed template more stably, mix the transferred supernatant according to the ratio of lysis product (DNA template): Buffer P2 = 5:1, and store at -20°C after mixing. Stable preservation varies with time and sample status. If the treated plant leaf supernatant is used for PCR amplification within one week, without adding Buffer P2, the supernatant should be stored at -20°C.

PCR Reaction Conditions

Cycle Step	Temperature	Time	Cycle number
Initial denaturation	94℃	5 min	1 cycle
Denaturation	94℃	10 sec	35 cycles
Annealing	50 - 65℃	20 sec	
Extension	72℃	1 min	
Final extension	72℃	5 min	1 cycle

[Note]:

a) Annealing temperature: Please refer to the theoretical Tm value of the primer. The annealing temperature can be set 2-5℃ lower than the theoretical value of the primer.

b) 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.

Cautions

1. When doing leaf experiments, it is recommended to use freshly collected leaf tissue. If it is a 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. The leaf tissue is suitable for young leaves. If it is a mature leaf, avoid using the tissue of the main vein of the leaf.
2. It is recommended to amplify the fragment within 1 kb in length for the best amplification efficiency.
3. When sampling, use a hole punch or knife to take a sample of suitable size. When the samples are different, the hole punch or knife needs to be cleaned every time before processing the sample.
4. For leaf tissue, it is recommended to take 1-10 mm leaves, too small leaf length will lead to low PCR amplification yield, too much will inhibit the PCR reaction, use the method of thermal cracking, mashing with a pipette tip, and crushing with a grinder to treat plant leaves. After treatment, 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 lab coats and disposable gloves for operation.
6. This product is for research use **ONLY** !