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Plant DNA Extraction Solution

Composition

Cat. No.	3203100
Plant DNA Extraction Solution	100 ml
Instructions	1

Storage

The reagents can be stored at room temperature (0~30℃) for up to 3 years without showing any reduction in performance.

Introduction

This product is suitable for extracting genomic DNA from plants. The extraction method is simple and fast, and multiple samples can be processed within 60 min. The obtained DNA can be used directly for Southern blotting, PCR, DNA cloning, and other related molecular biology experimental operations. Because of its precipitation method, all DNA fragments of different sizes can be precipitated, and it is also very effective for DNA extraction of apoptosis.

Equipment and Reagents to Be Supplied by User

1. β-mercaptoethanol, isopropanol, 75% ethanol and Buffer EX (or chloroform).
2. RNase A may be required.
3. 1.5 ml centrifuge tube.
4. Pipette and pipet tips.
5. Latex gloves, disposable masks and other protective items and paper towels.
6. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
7. Vortexer.
8. Water bath.
9. Mortar.

Cautions

1. If there are any precipitates in Plant DNA Extraction Solution, incubate to all the precipitates dissolved before use.
2. The extracted DNA may contain RNA contamination, but it does not affect PCR- related experiments. To remove RNA, add 1 μl RNase A (Simgen, Cat.No.8001001) with a final concentration of 40 μg/ml to the DNA solution and incubated at 37℃ for 30 min.

Preparation Before Use

1. If the centrifuge has refrigeration function, please set the temperature to 25℃.
2. Set the water bath temperature to 65℃ and incubate the Plant DNA Extraction Solution to 65℃ before use.

Protocol

1a. Liquid nitrogen crushing.

1) **Weigh 100~500 mg of tissue (chopped leaves/flowers/stems/roots/seeds) in a mortar, grind the tissue to powder form with liquid nitrogen.**

* Plant tissue must be ground to powder to fully destroy the plant cell walls, otherwise it will seriously affect the recovery efficiency of the final DNA.

* If the tissue particles do not reach powder form, continue with additional liquid nitrogen grinding.

2) **Add 1 ml Plant DNA Extraction Solution, 2 µl β-mercaptoethanol, continue grinding, incubate the mortar at 65℃ to slowly thaw the tissue, continue grinding to completely lyse the tissue.**

3) **Transfer 750 µl lysate into a 1.5 ml centrifuge tube and incubate at 65℃ for 30 min. Invert the tube several times every 8-10 min to facilitate the release of DNA.**

* For tissues with a lot of fiber such as grapes, the incubation time should be extended to 1 hour.

1b. External crushing.

1) **Place 100~500 mg of tissue (chopped leaves/flowers/stems/roots/seeds) in a mortar or homogenizer, add a small amount of Plant DNA Extraction Solution (100~200 µl), and grind vigorously until homogenized.**

2) **After grinding fully, add 800~900 µl Plant DNA Extraction Solution (and the total Plant DNA Extraction Solution is 1 ml before), 2 µl β-mercaptoethanol, continue grinding, so that the tissue is completely lysed.**

3) **Transfer 750 µl lysate into a 1.5 ml centrifuge tube and incubate at 65℃ for 30 min. Invert the tube several times every 8-10 min to facilitate the release of DNA.**

* For tissues with a lot of fiber such as grapes, the incubation time should be extended to 1 hour.

2. Add 750 µl Buffer EX (or chloroform), mix vigorously and centrifuge at 12000 rpm for 5 min.

* Buffer EX is low toxicity and non-volatile. It does not need to be operated in a fume cupboard and can be a perfect substitute for chloroform.

3. Transfer the supernatant (about 600 µl) into a new 1.5 ml centrifuge tube.

* DNA is in the upper layer. It is better to transfer less supernatant than to suck the middle protein.

4. Add 0.7 volume of isopropanol (about 420 µl), centrifuge at 12000 rpm for 10 min, discard the supernatant.

* The supernatant can be poured out directly but be careful not to pour away the pellet.

5. Add 1 ml 75% ethanol into the 1.5 ml centrifuge tube, vortex for a few seconds to resuspend DNA, centrifuge at 12000 rpm for 3 min, discard the supernatant.

6. Repeat step 5 once, then spin down and carefully discard the residual liquid with a 200 µl pipet tip.

7. Incubate at room temperature for a few minutes (about 10 min) to evaporate the residual ethanol. Add an appropriate amount (100 to 200 µl) of distilled water or TE buffer to dissolve the DNA pellet. The DNA can be used for various molecular biology experiments or stored at -20℃ for later use.

* Do not dry the DNA completely, as this will make it difficult to dissolve.

* If there are insoluble impurities in the DNA solution, centrifuge the DNA solution at 4℃,12000 rpm for 10 min and draw clear DNA solution for use.

* If DNA is extracted from fresh plant samples, it usually contains some RNA contamination, which does not affect PCR-related experiments. However, if it is necessary to completely remove RNA, refer to caution 2 to solve the problem.