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Plant/Fungus DNA Extraction Kit Instructions

Composition

Plant/Fungus DNA Extraction Kit Cat. No.	5 Preps 3200005	50 Preps 3200050	250 Preps 3200250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
β-Mercaptoethanol	20 μl	200 μl	1 ml
Buffer PD	6 ml	55 ml	260 ml
Buffer EX	5 ml	45 ml	225 ml
Buffer GP	4 ml	32 ml	160 ml
Buffer WA (Concentrate)	1.9 ml	12 ml	60 ml
Buffer WB (Concentrate)	1.5 ml	10 ml	50 ml
Buffer TE	1.2 ml	12 ml	60 ml
Instructions	1	1	1

Storage

The reagents can be stored at room temperature (0~30℃) for up to 2 years without showing any reduction in performance. if stored at 2-8℃, the validity period can be extended to more than 2 years (the reagents stored at 2~8℃ should be restored to room temperature before use).

Introduction

This product is suitable for extracting total DNA from fresh plant tissues or fungus with high polysaccharide and polyphenols at 100~500 mg (or dried plant tissues at 20~50 mg). After freezing with liquid nitrogen, ground the cells to broken, then add the lysis buffer to release genomic DNA. The protein, polysaccharide and other impurities of plant tissues were separated phase by Buffer EX. Add the supernatant containing DNA to the Spin Column, the DNA is bound to the Spin Column, and the remaining proteins and PCR inhibitors are filtered and removed. The DNA is washed by Buffer WA and Buffer WB and eluted with Buffer TE, which can be used for various molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, RNaseA storage solution may be required.
2. Liquid nitrogen, mortar.
3. 1.5 ml and 2 ml centrifuge tubes, pipette and tips.
4. Latex gloves, disposable masks and other protective items and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Vortexer
7. Water bath

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 Buffer PD and Buffer TE to 65℃.
3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label the reagent bottle and tick the box on the label to mark "Ethanol Added".

Protocol for Plants:

1. **Weigh 100~500 mg of fresh plant tissue or 20~50 mg of dried plant tissue (cut leaves/flowers/stems/roots/seeds) in a mortar or homogenizer, add 100~200 µl Buffer PD incubated to 65℃ and 2 µl β-mercaptoethanol, grind vigorously until homogenized.**

* For tissues with relatively high fiber content, increase the amount of tissue, grind the tissue into a powder form with liquid nitrogen, and then follow step 1, otherwise the recovery rate of DNA will be seriously affected.

2. **After grinding fully, add 800~900 µl Buffer PD incubated to 65℃ (the total volume of Buffer PD added with step 1 is 1 ml), continue grinding for 1 min, so that the tissue is completely lysed.**
3. **Transfer 800 µl lysate into a 2 ml centrifuge tube (not provided) and incubated at 65℃ for 30 min. Invert the tube several times every 5-10 min to facilitate the release of DNA.**

* For more fibrous tissues such as grapes, the incubation time can be appropriately extended to 1 hour.

* If DNA is extracted from a freshly obtained sample, it may be possible to extract some RNA in the tissue together, but the presence of RNA does not affect PCR-related experiments. For complete RNA removal, an additional 5 µl RNaseA (Simgen cat. no.8001001, 50 mg/ml, not provided) may be added to this step.

4. **Add 800 µl Buffer EX, mix vigorously, then centrifuge at 12000 rpm for 5 min.**
5. **Transfer the supernatant (about 600 µl) to a new 1.5 ml centrifuge tube.**
6. **Add an equal volume of Buffer GP to the supernatant and mix well.**
7. **Transfer half the volume of the mixture in step 6 (about 600 µl) to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12000 rpm for 30 sec.**
8. **Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube. Transfer all the remaining mixture in step 6 to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

9. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube, add 500 µl Buffer WA to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

10. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

11. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube, close the lid and centrifuge at 14,000 rpm for 1 min.**

* If the speed could not reach 14000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, as this will cause problems in downstream applications due to the residual ethanol in the eluate.

12. **Discard 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100-200 µl Buffer TE incubated to 65℃ into the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof lid, please change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the tube lid falling off.

13. **Discard the Spin Column. The eluted DNA can be used for various molecular biology experiments or stored at -20℃ for later use.**

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Protocol for Fungus:

1. **Add liquid nitrogen to immerse the fungus, first grind about 300~500 mg fungus into fine particles, wait for liquid nitrogen to evaporate, and then quickly grind the fungus to powder form.**

* The fungal cell wall is extremely tough, grind it to powder form with liquid nitrogen to achieve the purpose of fully destroying the cell wall, otherwise the final DNA recovery efficiency will be seriously affected.
* Liquid nitrogen should be added in time during fungus grinding to avoid fungus particles that are difficult to fully grind due to thawing.
* If the mycelium does not reach 300 mg (such as mold colonies), scrape off the entire colony to the mortar, and grind the colony to a powder form with liquid nitrogen.

2. **Add 1 ml Buffer PD incubated at 65℃ and 2 μl β-mercaptoethanol continue grinding for 1 min to completely lyse the fungus.**
3. **Transferred 800 μl lysate into a 2 ml centrifuge tube (not provided) and incubated at 65℃ for 30 min. Invert the tube several times every 5-10 min to facilitate the release of DNA.**

* If DNA is extracted from a freshly obtained sample, the RNA in the fungus (especially those with a high RNA content such as yeast) will usually be extracted together, but the presence of RNA does not affect PCR-related experiments. If the RNA is to be completely removed, an additional 5 μl RNaseA (Simgen cat. no.8001001, 50 mg/ml, not provided) may be added to this step.

4. **Add 800 μl Buffer EX, mix vigorously, then centrifuge at 12000 rpm for 5 min.**
5. **Transfer the supernatant (about 600 μl) to a new 1.5 ml centrifuge tube.**
6. **Add an equal volume of Buffer GP to the supernatant and mix well.**
7. **Transfer half the volume of the mixture in step 6 (about 600 μl) to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12000 rpm for 30 sec.**
8. **Discard the filtrate and place the Spin Column back into the 2 ml Collection Tube. Transfer all the remaining mixture in step 6 to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

9. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube, add 500 μl Buffer WA to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.

10. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube, add 600 μl Buffer WB to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

11. **Discard the filtrate. Place the Spin Column back into the 2 ml Collection Tube, close the lid and centrifuge at 14,000 rpm for 1 min.**

* If the speed could not reach 14000 rpm, centrifuge at full speed for 2 min.
* Do not omit this step, as this will cause problems in downstream applications due to the residual ethanol in the eluate.

12. **Discard 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100-200 μl Buffer TE incubated to 65℃ into the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 1 min.**

* If the centrifuge does not have a leak-proof lid, please change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the tube lid falling off.

13. **Discard the Spin Column. The eluted DNA can be used for various molecular biology experiments or stored at -20℃ for later use.**