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Vegetable Oil DNA Extraction Kit Instructions

Composition

Vegetable Oil DNA Extraction Kit Cat. No.	5 Preps 3109005	20 Preps 3109020
Connection Tubes	5	20
Extension Tubes	5	20
Spin Columns	5	20
2 ml Collection Tubes	5	20
Buffer OL	60 ml	240 ml
Carrier RNA	45 μL	180 μL
Proteinase K	0.6 ml	1.2 ml×2
Buffer P	120 ml	240 ml×2
Buffer WA (concentrate)	1.9 ml	6.5 ml
Buffer WB (concentrate)	1.5 ml	6 ml
Buffer TE	0.3 ml	1.2 ml
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Storage

1. Carrier RNA and Protease K can be transported at room temperature. Please store protease K and Carrier RNA at -20℃.
2. All the reagents and components can be stored for up to 2 years at room temperature (0~30℃). For longer storage, it is recommended to keep at 2~8℃.
3. The product stored at 2~8℃ should be restored to room temperature before use.

Technical Support

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Introduction

This kit uses a special extraction buffer, Buffer OL, and assisted digestion with proteinase K, which fully releases trace amounts of DNA from vegetable oil. With the aid of Carrier RNA, the column purification technology can efficiently adsorb trace amounts of small DNA fragments in various vegetable oils. The DNA adsorbed to the Spin Column is subjected to two wash buffers to remove protein residues and salts, and then eluted with Buffer TE, and the obtained DNA can be directly used for molecular biology experiments such as PCR detection.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 150 ml Erlenmeyer Flask, 50 ml centrifuge tube, 1.5 ml centrifuge tube, pipettes and tips.
3. Protective equipment such as latex gloves, disposable masks, and paper towels.
4. Centrifuge(s) (with rotor for 50 ml and 2 ml centrifuge tubes).
5. Thermostatic mixer or shaker, Vacuum manifold (Simgen Cat. No. A-100).
6. N-hexane may be required.

Preparation Before Use

1. If the centrifuge has refrigeration, set the temperature to 25℃.
2. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label the reagent bottle, and mark "Ethanol added" by ticking the box on the label.
3. Set the temperature of the thermostatic mixer or shaker to 56℃.

Protocol

1. **Take a clean 150 ml Erlenmeyer Flask, add 10 ml vegetable oil (or 10 g if solid oil), 10 ml Buffer OL, 7.5 µl Carrier RNA, and 100 µl Proteinase K, cork the Erlenmeyer Flask, and incubate for 1 hour at 200 rpm at 56℃ in a thermomixer or shaker (solid oil bath time can be extended to 2 hours).**

* If solid vegetable oil does not melt completely at 56°C, add 10 ml n-hexane and incubate at 56°C for 1 hour at 200 rpm.

2. **Pour all the liquid from the Erlenmeyer flask into a clean 50 ml centrifuge tube and centrifuge at 4500 rpm for 10 min.**
3. **Transfer the lower aqueous phase (about 10 ml) into a clean 50 ml centrifuge tube, taking care not to transfer the oil phase or the white precipitate in between, so as not to affect the subsequent DNA extraction.**
4. **Add 2 times volume of Buffer P, vortex to mix well.**

* e.g. Transfer 10 ml lower aqueous phase, need to add 20 ml Buffer P.

5. **Insert the Connection Tube into the socket of the Vacuum manifold, then insert the Spin Column on the Connection Tube, and finally insert the extension tube on the Spin Column (see the instructions of the Vacuum manifold for details). Transfer 20 ml mixture from step 4 into the extension tube of the Spin Column and turn on the negative pressure so that all the liquid is filtered through the Spin Column.**

* To avoid cross-contamination, please do not reuse the Connection Tube.

* To avoid cross-contamination, do NOT insert the Spin Column directly into the socket of the Vacuum manifold.

6. **Turn off the negative pressure, transfer all the remaining mixture in step 4 into the extension tube of the Spin Column, turn on the negative pressure, and let the liquid all filter through the Spin Column.**
7. **Turn off the negative pressure, remove and discard the Spin Column extension tube, add 600 µl Buffer WA to the Spin Column, and turn on the negative pressure to drain the solution in the Spin Column.**

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

8. **Turn off the negative pressure, add 800 µl Buffer WB to the Spin Column and turn on the negative pressure to drain the solution in the Spin Column.**

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

9. **Turn off the negative pressure, remove and discard the Connection Tube when the negative pressure is gone, place the Spin Column into a 2 ml Collection Tube provided with the kit, close the cap, and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, otherwise the ethanol may mixed in the purified nucleic acid.

10. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 30~50 µl Buffer TE to the Spin Column center, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**
11. **Discard the Spin Column and the eluted DNA can be used immediately for PCR testing or stored at -20°C for later use.**