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Trace DNA Extraction Kit Instructions

Composition

Trace DNA Extraction Kit Cat. No.	5 Preps 3102005	50 Preps 3102050
Spin Columns	5	50
2 ml Collection Tubes	5	50
1.5 ml Collection Tubes	5	50
Proteinase K	120 µl	1.2 ml
Carrier RNA	40 µl	300 µl
Buffer AT	1.5 ml	15 ml
Buffer SL	2 ml	15 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WB (concentrate)	1.5 ml	10 ml
Buffer TE	1.2 ml	12 ml
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Storage

1. Proteinase K and Carrier RNA should be stored at -20℃.
2. Other reagents and items, if stored at room temperature (0~30℃), can maintain no significant change in performance within 2 years; If the product is stored at 2~8℃, the validity period of the product can be extended to more than 2 years (products stored at 2~8℃ should be restored to room temperature before use).

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This product is suitable for the extraction of total DNA (including genomic DNA, mitochondrial DNA and possibly viral DNA) from trace amounts of human or animal tissue. After the animal tissue is digested by proteinase K, the DNA is bound to the Spin Column, the degraded proteins and PCR inhibitors are filtered out, and the DNA is washed by Buffer WA and Buffer WB and eluted with Buffer TE, which can be used in various molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol, 1 M DTT (Dithiothreitol).
2. 1.5 ml centrifuge tube.
3. Pipettes and tips.
4. Disposable gloves and protective supplies and paper towels.
5. Microcentrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Constant temperature shaker or water bath and vortexer.

Preparation before use

1. If the centrifuge has a refrigeration function, set the temperature to 25℃.
2. Set the constant shaker temperature or water bath temperature to 56℃ and 70℃ and incubate Buffer AT and Buffer TE to 56℃.
3. Add Absolute ethanol to Buffer WA and Buffer WB according to the instructions on the bottle label and tick the box on the label to mark "Ethanol Added".

Protocol

1A. Dry blood spots:

Take 1 to 3 pieces of dry blood spot with a diameter of 3 mm and put them into a 1.5 ml centrifuge tube (not provided) with 180 µl Buffer AT and 20 µl Proteinase K.

1B. Cigarette butt:

Strip 1 cm^2 of outer paper from the cigarette butt or filter tip, cut it into pieces, and put it into 1.5 ml centrifuge tube (not provided) with 250 µl Buffer AT and 20 µl Proteinase K.

1C. Hair root

Take 0.5 to 1 cm of hair roots and place it into a 1.5 ml centrifuge tube (not provided) with 180 µl Buffer AT, 20 µl Proteinase K, and 15 µl 1 M DTT.

1D. Hair

Cut the hair into 0.5 to 1 cm pieces and place in a 1.5 ml centrifuge tube (not provided) with 180 µl Buffer AT, 20 µl Proteinase K, and 15 µl 1 M DTT.

1E. Nail

Cut the nail into small particles and put them into a 1.5 ml centrifuge tube (not provided) with 180 µl Buffer AT, 20 µl Proteinase K and 15 µl 1 M DTT.

1F. Clothing with blood, saliva, sperm spot

Cut off about 0.5 cm^2 of clothes, cut it into pieces, and place it into 1.5 ml centrifuge tube (not provided) with 250 µl Buffer AT and 20 µl Proteinase K (if sperm is present, 15 µl 1 M DTT should be added).

1G. Serum or plasma

Take 200 µl serum or plasma into 1.5 ml centrifuge tube (not provided) (if less than 200 µl, add normal saline to 200 µl) and add 20 µl Proteinase K.

2. **Vortex to mix well and incubate at 900 rpm for 1 h in a constant temperature shaker at 56°C.**

* If using a water bath, vortex several secs every 10 min to help lysing.
* Hair, nails and other specimens that are not easily lysed can be appropriately extended for the water bath time (such as overnight digestion) until they are completely lysed.

3. **Add 5 µl Carrier RNA and 250 µl Buffer SL and vortex for about 15 sec to mix. Incubate at 900 rpm for 10 min in a constant temperature mixer at 70°C.**
4. **Centrifuge at 14,000 rpm for 1 min.**

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

5. **Transfer 400 µl supernatant to a new clean 1.5 ml centrifuge tube (not provided), add 320 µl absolute ethanol, vortex to mix well. Spin down the solution to the bottom of the tube.**
6. **Transfer the solution in step 5 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.**

* Be careful not to dip the solution onto the nozzle of Spin Column. Otherwise, it cannot completely wash the Spin Column in subsequent washing steps.

7. **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 the Buffer WA.
* The filtrate does not need to be completely discarded. To avoid contamination of the centrifuge by the filtrate adhering to the mouth of the collection tube, invert the 2 ml Collection Tube and slap once on a paper towel.

8. **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.

9. **Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube, close the lid 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, or the purified nucleic acid maybe mixed with ethanol and affect the subsequent PCR.

10. **Discard 2 ml Collection Tube, place the Spin Column in a 1.5 ml Collection Tube, add 25~50 µl Buffer TE incubated at 56℃ into the Spin Column, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the volume of Buffer TE added is less than 25 µl, the concentration of eluted DNA may no longer increase.

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