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Blood Spots DNA Extraction Kit

Composition

Blood Spots DNA Extraction Kit Cat. No.	5 Preps 3012005	50 Preps 3012050	250 Preps 3012250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
1.5 ml Collection Tubes	5	50	250
Proteinase K	120 µl	1.2 ml	1.2 ml×5
Buffer AT	1.5 ml	15 ml	75 ml
Buffer SL	1.5 ml	15 ml	75 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

1. Proteinase K should store at -20℃.
2. All other reagents can be stored at room temperature for up to 2 years without showing any reduction in performance and would be stable more than 2 years if stored at 2-8℃ (the product 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 extracting total DNA (including genomic DNA, mitochondrial DNA, and possible viral DNA) from 1-5 pieces of 0.5cm^2 dried blood spots samples. After digestion by Proteinase K, the released DNA will be bound to the Spin Column, the degraded proteins and PCR inhibitors will be filtered out, and the DNA will be 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.
2. 1.5 ml centrifuge tubes.
3. Pipettes and tips.
4. Disposable gloves and protective supplies and paper towels.
5. Centrifuge(s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
6. Thermostatic mixer, 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 thermostat mixer, shaker, or water bath temperature to 56℃ and 70℃ and incubate the Buffer AT and Buffer TE to 56℃.
3. Add absolute ethanol to Buffer WA and Buffer WB according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol added".

Protocol

1. **Cut about 0.5 $cm^2$of dry dried blood spots into pieces and put it into 1.5 ml centrifuge tube (not provided).**

* If conditions permit, collect 3-5 pieces of 0.5 cm^2 dried blood spots to increase the yield of the final DNA.

2. **Add 250 µl Buffer AT incubated at 56℃ and 20 µl Proteinase K, vortex to mix well, incubate at 56℃ at 900 rpm for 1 h.**

* If using a water bath, vortex several seconds every 10 min to aid lyse.

3. **Add 250 µl Buffer SL and vortex for about 15 sec to mix. Incubate at 70℃ or shake at 900 rpm for 10 min.**

* If the sample is less than 0.5 cm^2 or has been stored for a long time (more than half a year), the DNA content is too low, or the DNA has degraded, it is recommended to add 1 µl Carrier RNA (Simgen Cat. No.4003101) to improve the efficiency of DNA recovery.

4. **Centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at 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 mixture 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 add the solution on the edge of the tube mouth of the Spin Column.

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.**

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

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 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 is less than 25 µl, the eluent may not be able to permeate the silica gel membrane, and the efficiency of DNA recovery may be reduced.

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