Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

Sperm DNA Extraction Kit Instructions

Composition

Sperm DNA Extraction Kit Cat. No.	5 Preps 4202005	50 Preps 4202050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Proteinase K	120 µl	1.2 ml
Buffer SP	100 µl	1 ml
Buffer SL	1.2 ml	12 ml
Buffer WA (concentrate)	1.9 ml	12 ml
Buffer WB (concentrate)	1.5 ml	9.5 ml
Buffer TE	1.2 ml	12 ml
Instructions	1	1

Storage

1. Buffer SP and Proteinase K should be stored at -20℃.
2. If other reagents and items are stored at room temperature (0~30℃), they can keep their performance unchanged for 2 years, and if stored at 2~8℃, they can extend the validity period to more than 2 years (the product stored at 2~8℃ should be restored to room temperature before use).

Technical Support

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

Introduction

This product is suitable for total DNA extraction from ≤ 200 µl fresh or frozen sperm. The sperm sample is digested by proteinase K to release DNA, ethanol is added to promote DNA binding to the Spin Column, and the degraded protein and PCR inhibitors are filtered and removed.

Equipment and reagents to be supplied by users

1. Absolute ethanol
2. 1.5 ml centrifuge tubes and pipette and tips (to avoid contamination between samples, use pipette tips with filters)
3. Disposable gloves and protective equipment and tissues
4. Microcentrifuge (s) (rotor with 1.5 ml and 2 ml tubes)
5. Water bath and vortexer
6. PBS solution may be required

Preparation before use

1. If the centrifuge has refrigeration function, set the temperature to 25℃.
2. Set the water bath temperature to 56℃ and incubate 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, tick the box on the label to mark "Ethanol Added".

Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

Protocol

1. **Add 20 µl Proteinase K and 200 µl liquefied sperm to a 1.5 ml centrifuge tube.**

* If DNA is extracted from less than 200 µl sperm, add PBS solution to bring the sample to 200 µl.

2. **Add 15 µl Buffer SP and 200 µl Buffer SL，vortex for 15 sec to mix well。**

* Do not add Proteinase K directly to Buffer SL.

3. **Incubate at 56℃ for 10 min. Spin down the solution on the cap to settle to the bottom of the tube.**
4. **Add 200 µl absolute ethanol and vortex for about 15 sec. Spin down the solution on the cap to settle to the bottom of the tube.**

* For sperm samples with high sperm content, precipitation may occur after the addition of absolute ethanol, and continuous vortex should be carried out until the precipitation is dispersed, otherwise the Spin Column may be blocked in subsequent steps.

5. **Transfer the solution from step 4 to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**

* Be careful not to get the solution on the edge of the Spin Column orifice, so that subsequent washing steps fail to wash the Spin Column.

6. **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 12,000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WA.
* The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the centrifuge tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

7. **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 12,000 rpm for 30 sec.**

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

8. **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 centrifuge does not reach 14,000 rpm, centrifuge at its full speed for 2 min.
* Do not omit this step, otherwise the subsequent PCR effect may be affected due to the ethanol mixed in the purified DNA.

9. **Discard the 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100~200 µl Buffer TE incubated to 56℃ to the center of the Spin Column, close the lid, incubate at room temperature for 1 min, and centrifuge at 12000 rpm for 30 sec.**

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm centrifugation for 1 min to avoid the tube lid coming off and damaging the centrifuge.
* Increasing the volume of Buffer TE can improve the elution efficiency of DNA, but will reduce the concentration of DNA, but if the volume of Buffer TE is less than 100 µl, the DNA yield may be reduced due to insufficient infiltration of the silica membrane.

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