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

Rapid Universal Genomic DNA Extraction Kit Instructions

Composition

Rapid Universal Genomic DNA Extraction Kit Cat. No.	5 Preps 3105005	50 Preps 3105050	250 Preps 3105250
Spin Columns	5	50	250
2 ml Collection Tubes	5	50	250
Proteinase K	120 μl	1.2 ml	1.2 ml×5
Buffer GE	1.8 ml	18 ml	80 ml
Buffer PE	3 ml	30 ml	130 ml
Buffer WB (concentrate)	3 ml	19 ml	50 ml×2
Buffer TE	3 ml	30 ml	150 ml
Instructions	1	1	1

Storage

1. Store Proteinase K at -20℃.
2. If other reagents and items are stored at room temperature (0~30℃), they can keep the performance unchanged for 2 years, and if stored at 2~8℃, the validity period can be extended to more than 2 years.

Technical Support

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

Introduction

This product uses a unique lysis buffer combined with proteinase K to digest various types of biological samples to release DNA, supplemented by original extraction technology to quickly remove proteolytic products, lipids, polysaccharides, phenolic derivatives, pigments and other impurities in biological samples. Specially designed Spin Columns selectively bound large DNA fragments from the aqueous phase, while small nucleic acids are selectively filtered out. The bounded DNA on the Spin Column only needs to be washed 1~2 times with one wash buffer to obtain high-purity genomic DNA. It is suitable for PCR, Southern blotting analysis, RAPD, RFLD and other molecular biology experiments.

Equipment And Reagents to Be Supplied by Users

1. Absolute ethanol
2. 1.5 ml centrifuge tubes, pipettes, and tips (pipette tips with filters are recommended to avoid contamination between samples).
3. Disposable gloves and protective equipment and tissues
4. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes)
5. Water bath and vortexer
6. RNase A (50 mg/ml, Simgen Cat. No. 8001001）

Preparation before use

1. If the centrifuge has refrigeration, 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 WB according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".
4. When the room temperature is lower than 15℃, the reagent should be observed for precipitation before using Buffer GE and Buffer PE, and if there is precipitation, incubate at 56℃ until the precipitate is dissolved before use.

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

Samples are prepared before use

【Genomic DNA Extraction from Animal Tissues】

1. **Cut 20~50 mg of animal tissue with a scalpel, chop the tissue into a homogenate with the tip of the knife, and transfer it into a clean 1.5 ml centrifuge tube.**

* For the following tissues, add liquid nitrogen to grind to the powder form, put the mortar in a 56℃ water bath, continue grinding for 1 min when the powder begins to thaw, add 200 µl Buffer TE and 300 µl Buffer GE, continue to grind for 30 sec to mix well, transfer all the homogenate to a clean 1.5 ml centrifuge tube before entering step 3.
  DNase-rich tissues of the pancreas, spleen, thymus, lymph, etc.
  Collagen-rich skin, muscle health and other tissues.
  Keratin-rich tissues or hard tissues such as bones, etc.

2. **Add 150 µl Buffer TE and 300 µl Buffer GE, vortex and shake for 30 sec to mix well.**
3. **Add 20 µl Proteinase K, incubate at 56℃ for 10 min.**

* If you want to improve the yield of DNA, you can extend the water bath time until the tissue is completely lysed before proceeding to the next step.

4. **Add 500 µl Buffer PE, shake vigorously for 5~10 times, then vortex for 30 sec to mix well, and centrifuge at full speed (≥12,000×g) for 1 min.**

[Genomic DNA Extraction from Plant Tissues]

1. **Weigh an appropriate amount of fresh plant tissue in the table below (if freeze-dried tissue is selected, the amount of tissue should be halved), cut into small pieces and put it in a mortar, add liquid nitrogen, freeze the tissue completely, and grind it quickly and vigorously to powder. Liquid nitrogen should be added intermittently to prevent the tissue from thawing, then place the mortar in a 56℃ water bath until the sample powder has just begun to thew. Add 300 µl Buffer TE and continue grinding for 30 sec to mix.**

Sample Type	Dosage
Plant flowers or leaves	100-200 mg
Plant roots, stems, seeds	≤ 240 mg

* Sample grinding should be sufficient, otherwise the yield of genomic DNA will be seriously affected.

2. **Transfer 200 µl ground homogenate to a 1.5 ml centrifuge tube, and if the homogenate volume is less than 200 µl, add Buffer TE to 200 µl.**

* If the homogenate is thick and cannot be transferred, it means that the sample is too much, you can add 200 µl Buffer TE to continue grinding and mixing, and then transfer 200 µl homogenate to the next step.

3. **Add 300 µl Buffer GE and 20 µl Proteinase K and immediately vortex for 1 min to mix well. incubate at 56℃ for 10 min.**

* Do not add Proteinase K directly to Buffer GE.
* Samples such as fiber-rich roots/stems or starch and protein-rich seeds can be extended to 30 min.

4. **Add 500 µl Buffer PE, shake vigorously for 5~10 times, then vortex for 30 sec to mix well, and centrifuge at full speed (≥12,000×g) for 5 min.**

[Genomic DNA Extraction from Cultured Cells, Lymphocytes, Whole Blood, Bone Marrow, And Bones]

1a. Animal suspension culture cells or freshly isolated single-cell suspension of animal tissues

Centrifuge at 300×g for 5 min to collect approximately 5×10^6 cells, discard the supernatant, and add 200 µl Buffer TE to suspend the cells.

1b. Adherent cultured cells

Discard the culture supernatant, add trypsin and suspend the cells, centrifuge at 300×g for 5 min to collect about 5×10^6 cells. Discard the supernatant and add 200 µl Buffer TE to suspend the cells.

1c. Lymphocytes

Collect approximately 5×10^6 lymphocytes, control the cell suspension at 200 µl, and if the cell suspension is less than 200 µl, add Buffer TE to a final volume of 200 µl.

1d. Whole blood or bone marrow

Collect 200 µl anticoagulated whole blood or bone marrow (EDTA anticoagulation).

1e. Bone

Take 50-100 mg of minced bones, transfer them into a mortar and grind them into a homogenate, add 300 µl Buffer TE, and continue to grind for 30 sec to mix. Transfer 200 µl homogenate into a 1.5 ml centrifuge tube.

2. Add 300 µl Buffer GE and 20 µl Proteinase K. Immediately vortex for 30 sec to mix well.

* Do not add Proteinase K directly to Buffer GE.

3. Incubate at 56℃ for 10 min.

4. Add 500 µl Buffer PE, shake vigorously for 5~10 times, then vortex for 30 sec to mix well, and centrifuge at full speed (≥12,000×g) for 1 min.

【Genomic DNA Extraction from Bacteria】

1. Collect 3~5 ml bacterial culture with a 1.5 ml centrifuge tube, add 100 µl Buffer TE, and vortex to fully suspend the bacteria. Add 100 µl lysozyme solution (Simgen Cat. No. 8009500, not provided), vortex for about 15 sec to mix, incubate at 37℃for 30 min.

* Some divalent cations can inhibit the activity of lysozyme, if the bacterial medium contains divalent cations (such as MRS medium, etc.), a washing step should be added after centrifugation to collect the bacteria: add 1 ml deionized pure water, vortex to suspend bacteria, centrifuge at 12,000×g for 30 sec, discard the supernatant, add 100 µl Buffer TE, and vortex to fully suspend the bacteria.
* Preparation method of lysozyme solution: 100 mg/ml lysozyme solution is prepared by adding 1 ml deionized pure water per 100 mg lysozyme.
* Lysozyme solution will seriously reduce the lyse efficiency after freezing and thawing, please try to use freshly prepared or freeze-thaw lysozyme solution no more than once.

2. Add 300 µl Buffer GE and 20 µl Proteinase K. Immediately vortex for 30 sec to mix well.

* Do not add Proteinase K directly to Buffer GE.

3. Incubate at 56℃ for 10 min.

4. Add 500 µl Buffer PE, shake vigorously for 5~10 times, then vortex for 30 sec to mix well, and centrifuge at full speed (≥12,000×g) for 1 min.

[Genomic DNA Extraction from Yeast]

1. Collect 5~10 ml yeast cultured overnight and discard the supernatant. Add 100 µl Buffer TE, do not discard the tip, pipette the pellet directly with the tip, and transfer it into the mortar. Pour liquid nitrogen to soak the yeast solution (the yeast liquid will immediately coagulate into a lump when it touches the liquid nitrogen) and grind vigorously until the yeast mass is in powder form.

* If yeast is collected with a 1.5 ml centrifuge tube, the enrichment cells can be centrifuged multiple times at 12,000 rpm for 30 sec.
* If the bacterial block fails to be ground into powder, liquid nitrogen should be added to continue grinding, otherwise the recovery efficiency of the final DNA will be seriously affected.
* In the absence of liquid nitrogen, the cell wall can be fully destroyed by direct grinding for about 15 min, and if the liquid is evaporated during this process, add 100 µl Buffer TE to continue grinding.

2. When the powdered yeast begins to thaw, add 100 µl Buffer TE and 2 µl RNase A (Simgen Cat. 8001001), continue grinding several times, transfer the lysate to a 1.5 ml centrifuge tube, and incubate at 56℃ for 5 min.

* If the lysate is less than 200 µl, add Buffer TE to 200 µl.

3. Add 300 µl Buffer GE and 20 µl Proteinase K, vortex for 30 sec to mix well, incubate at 56℃ for 20 min. During the incubating, invert the centrifuge tube several times every 2~3 min to help release DNA.

* Do not add Proteinase K directly to Buffer GE.

4. **Add 500 µl Buffer PE, shake vigorously for 5~10 times, then vortex for 30 sec to mix well, and centrifuge at full speed (≥12,000×g) for 1 min.**

[Genomic DNA Extraction from Stool]

1. **Collect 30~50 mg of stool with a 1.5 ml centrifuge tube, add 150 µl Buffer TE, and vortex until all the stool particles are suspended.**

* If the stool is in liquid form, transfer 50 µl stool directly, if DNA is extracted from mouse stool, add 150 µl Buffer TE and use a grinding rod (Simgen Cat. No. D-050, not provided) grinding stool pellets.

2. **Add 300 µl Buffer GE and 20 µl Proteinase K. Immediately vortex for 30 sec to mix well.**

* Do not add Proteinase K directly to Buffer GE.

3. **Incubate at 56℃ for 10 min.**
4. **Add 500 µl Buffer PE, shake vigorously for 5~10 times, then vortex for 30 sec to mix well, and centrifuge at full speed (≥12,000×g) for 1 min.**

Follow The Same Steps

5. **Transfer all the supernatant and add it to a Spin Column (the Spin Column is placed in a 2 ml Collection Tube), close the lid, and centrifuge at 12,000×g for 30 sec.**

* It is better to transfer less of the supernatant than to transfer the interphase precipitate, which can seriously affect the purity of the final DNA.

6. **Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube, add 800 µl Buffer WB to the Spin Column, close the lid, and centrifuge at 12,000×g for 30 sec.**

* 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 collection tube, you can slap the 2 ml Collection Tube upside down on a paper towel once.

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

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 300 µl Buffer WB to the Spin Column, close the lid, and centrifuge at full speed (≥ 12,000 ×g) for 1 min.**
8. **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 Spin Column center, close the lid, incubate at room temperature for 1 min, and centrifuge at 12,000×g for 30 sec.**

* Note that when taking out the Spin Column, do not let the filtrate touch the bottom of the Spin Column, if the Spin Column is contaminated with filtrate, please discard the filtrate and put the Spin Column back to the 2 ml Collection Tube, centrifuge at full speed for 1 min, and then take out the Spin Column for this procedure.

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8,000×g for 1 min to avoid the tube lid coming off and damaging the centrifuge.

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