Nucleic Acid Extraction Kit Ⅰ (Magnetic Beads Method)

Principle

This kit uses a guanidine salt solution to release nucleic acids from samples, then adopts specific magnetic beads to adsorb nucleic acids, removes residual proteins and salts with wash buffer at the same time, and finally elutes the nucleic acids adsorbed on the specific magnetic beads with elution buffer under high temperature conditions [1-2]

Specification

100 Reactions/Kit

Usage

It is used for the extraction, enrichment and purification of nucleic acids, and the processed products are used for PCR detection.

Main Components

Components	Specification（100 Reactions/Kit）
Lysis Buffer	60 mL×1 bottle
Wash Buffer	100 mL×1 bottle
Elution Buffer	10 mL×1 bottle
Proteinase K	1 mL×3 tubes
Magnetic Beads	1 mL×1 tube
Instructions	1 copy

Note: Components from kits of different lot numbers cannot be interchanged, and the indicated reagent volume of each component is the minimum aliquot volume.

Storage conditions and shelf life

1.Store the kit at 10-30°C with a validity period of 24 months.

2.Freezing of the kit is prohibited, and strong light exposure should be avoided.

3.The product lot number and validity period are indicated on the outer package of the product.

Applicable Instruments

This kit is compatible with Biori's Automatic Nucleic Acid Extractor and other automated nucleic acid extraction equipment verified to meet the reagent extraction conditions.

Sample Preparation

1.Sample treatment: For sample types such as cell suspension and cell supernatant—for cell suspension, it is recommended to centrifuge at 1000×g for 5 minutes to pellet cells and take the supernatant for nucleic acid extraction; cell supernatant can be used for direct extraction. If sample concentration is required, first centrifuge at 1000×g for 5 minutes to pellet cells, transfer the supernatant to a new centrifuge tube, centrifuge at 18000×g for 30 minutes, discard part of the supernatant and retain about 400 μL for the next experiment.

If the test sample is an upstream intermediate sample in the purification process of biological products, it may contain a high DNA content. To ensure the accuracy of detection and make the detected value of the sample fall within the linear range of the standard curve, samples with high DNA content can be appropriately diluted with DNA dilution buffer or 1×PBS (pH7.4, $Ca^{2+}$and Mg^{2+}free) before purification; the purified samples can also be diluted with dilution buffer after purification and then subjected to residual DNA detection. It is generally recommended to dilute samples with high DNA content by 100 or 1000 times. If the sample is diluted, use the dilution buffer as the negative control.

2.If the sample is in powder form, dissolve the powder sample with dilution buffer before the next operation; or first dissolve the powder sample with an appropriate reagent to prepare a high-concentration solution, then dilute it with dilution buffer for the next operation. It is generally recommended to dilute the powder sample to 10 mg/mL or 100 mg/mL.

3.pH requirement: In general, the pH of intermediate samples in the purification process of biological products is neutral. If the pH of the sample is <5 or >9, the sample purification effect will be affected. Therefore, test the pH of the sample before sample treatment, and adjust the pH of the sample to neutral (pH 6.0-8.0) with 2M hydrochloric acid or sodium hydroxide before purification.

4.Parallel sample treatment: To ensure the accuracy of results, it is recommended to perform three parallel DNA extraction and detection for each sample.

Test Method

Before use, make the reagents in each bottle/tube settle at the bottom as much as possible.

A. Manual Operation Procedure

1.Sample addition: Add 600 μL Lysis Buffer, 10 μL Magnetic Beads (mix well before aspiration), 10 μL Proteinase K, internal standard (if applicable, refer to the requirements of the amplification kit instructions), and 100 μL-400 μL sample or treated sample solution into a 1.5 mL nuclease-free centrifuge tube in sequence, vortex and mix thoroughly, incubate at 37℃ for 2 min, then centrifuge at low speed for 2 s to spin the sample from the tube wall or cap to the bottom of the tube;

2.Perform magnetic adsorption on the above mixed solution after centrifugation for 2 min, discard the supernatant and remove the magnetic field;

3.Add 1 mL Wash Buffer and 20 μL Proteinase K, vortex and mix thoroughly, centrifuge at low speed for 2 s, then perform magnetic adsorption for 2 min, discard the supernatant and remove the magnetic field;

4.Add 100 μL Elution Buffer, vortex and mix thoroughly, centrifuge at low speed for 2 s, then elute at 80℃ in a Dry Bath Incubator for 5 min;

5.After magnetic adsorption for 1 min, take the extracted or purified product for subsequent experiments.

※ The magnetic adsorption time is related to the magnetic strength of the magnetic plate. If magnetic beads remain, appropriately increase the magnetic adsorption time, and the supernatant must be completely discarded;

※ The recommended low-speed centrifugation speed is not more than 3000 rpm/min, which is to spin the sample from the tube wall or cap to the bottom of the tube. High-speed centrifugation is strictly prohibited during the experiment.

B. Automated Extraction Method (Taking Biori's Automatic Nucleic Acid Extractor as an example)

1.Prepare 96 well Deep Well Plates and Magnetic Rod Sleeves matching the nucleic acid extraction equipment;

2.Reagent aliquoting: Aliquot the reagents into the corresponding wells of the 96 well Deep Well Plate according to steps 3-7 below;

Note: The position of the heating module may vary with different equipment, and the position of reagent aliquoting should be adjusted according to the actual situation.

3.Add 600 μL Lysis Buffer per well, 10 μL Magnetic Beads per well, 10 μL Proteinase K per well, internal standard (if applicable, refer to the requirements of the amplification kit instructions), and 100 μL-400 μL liquid sample per well to the wells in columns A2-H2 and A8-H8 of the 96 well Deep Well Plate respectively;

4.Add 1000 μL Wash Buffer per well and 20 μL Proteinase K per well successively to the wells in columns A4-H4 and A10-H10 of the 96 well Deep Well Plate;

5.Add 100 μL Elution Buffer per well to the wells in columns A6-H6 and A12-H12 of the 96 well Deep Well Plate;

6.Place the 96 well Deep Well Plate with added samples into the applicable automatic nucleic acid extraction equipment;

7.Take out the Magnetic Rod Sleeves and insert them into the appropriate position of the automatic nucleic acid extraction equipment;

8.Set the extraction program and run it according to the following parameters:

Step	Item	Well Position	Volume	Mixing Frequency	Mixing Time	Standing Time	Magnetic Adsorption Times	Drying Time	Lysis Temperature	Elution Temperature
1	Lysis	2	1000 μL	Fast	180 s	0 s	5	0 s	40℃	0℃
2	Washing	4	1000 μL	Medium	60 s	0 s	3	0 s	0℃	80℃
3	Elution	6	100 μL	Fast	180 s	0 s	3	0 s	0℃	80℃
4	Beads	4	1000 μL	Fast	10 s	0 s	0	0 s	0℃	0℃

※ The magnetic adsorption time is related to the magnetic strength of the magnetic rod. If magnetic beads remain, appropriately increase the magnetic adsorption time.

After the completion of the automated extraction program, transfer the extracted products in the wells of columns 6 and 12 into clean 1.5 mL nuclease-free centrifuge tubes. Store the extracted products at -20℃; if used for immediate detection, store at 2-8℃.

Limitations of the Test Method

Sample volume: The maximum volume of the extracted sample shall not exceed 400 μL.

Product Performance Indicators

High efficiency and rapidity: The operation time for on-machine extraction with this product is only about 10 minutes.

Precautions

1.Read the instruction manual carefully before operation and strictly follow the manual for experimental operation.
2.Avoid conducting experiments in harsh environments (such as environments with high-concentration corrosive gases and dust including 84 disinfectant, sodium hypochlorite, acid, alkali or acetaldehyde). Laboratory disinfection should be carried out after the experiment.
3.The product extracts nucleic acids including DNA and RNA. All used vessels, pipettes, etc. must be dedicated. Disposable consumables such as centrifuge tubes and pipette tips must be DNase/RNase-free; pipette tips shall not be shared between different samples to avoid cross-contamination.
4.The components of this kit contain chemical reagents, preservatives, etc., which have certain chemical hazards and shall not contact the skin or mucous membranes. If any reagent contacts the skin or mucous membranes, immediately rinse and disinfect the affected area extensively with a large amount of clean water. All samples and used kits shall be regarded as potentially infectious substances, and their disposal shall be in accordance with the regulations of the local government and relevant countries. Sample treatment shall be carried out in a clean bench or biosafety cabinet.
5.Use each component of the kit within the validity period indicated on the outer package. Seal the remaining reagents in a timely manner to prevent reagent volatilization, and store them at the specified ambient temperature.
6.If there is crystallization in the reagent before use, it can be properly heated until the crystals are completely dissolved before use.
7.If you have any questions during the use of the kit, please contact the company's sales or technical support.

References

1.Peter R Levi son, Stephen E Badger, Jon Dennis, et al. Recent developments of magnetic beads for use in nucleic acid purification[J].Chromatography A, 1998, 81:107.
2.Berensmeier S, Magnetic particles for the separation and purification of nucleic acids[J], Applied Microbiology and Biotechnology, December 2006, Volume 73, Issue3, 495–504.

Disclaimer

In all cases, the company's liability for this product is limited to the value of the product itself.