Universal Genomic DNA Extraction Kit (Spin Column)

Instruction for Use (V1.1)

[REF] UBE-S00250J UBE-S002200J

[Specification] 50 preps/kit, 200 preps/kit

[Research Use]

Ultrassay Genomic DNA Extraction Kit (Spin Column) is used for quick and simple genomic DNA extraction from various samples including blood, cells and tissues.

[Test Principles]

The spin column is made of new type silica membrane which can bind DNA optimally on given salt and pH conditions. The extraction process does not require phenol-chloroform extraction. The residual protein, salt and other impurities are removed through rapid and sufficient washing steps.

The extracted genomic DNA has large fragments, high purity, and good stability. It can be directly used for PCR, enzyme digestion and hybridization experiments.

[Kit Contents]

No.	Content	50 preps	200 preps	No.	Content	50 preps	200 preps
1	Buffer DA	15ml	50ml	6	Proteinase K	1.2ml	60ml
2	Buffer DLT	15ml	50ml	7	Bind DNA Spin Column	50pcs	4x50pcs
3	Buffer DW1	13ml	52ml	8	2ml Collection Tube	50pcs	4x50pcs
4	Buffer DW2	12ml	50ml	9	1.5ml Microcentrifuge Tube	50pcs	4x50pcs
5	Buffer EB	15ml	60ml				

[Storage Conditions and Shelf Life]

All the buffers can be stored dry at room temperature (15-25°C) for up to 12 months. Please check whether there is crystallization or precipitate in Buffer DA and Buffer DLT before use. If any, please re-dissolve Buffer DA and Buffer DLT in a 37°C water bath.

[Acceptable Specimens]

Blood, Cells and Tissues.

[Test Procedures]

Before use, add anhydrous ethanol to Buffer DW2 and Buffer DW2 indicated on the bottle label.

1. Processing the sample:

a. Mammalian blood: 200 µl of fresh or anticoagulated blood can proceed the next steps directly. if the blood is less than 200µl, adjust the blood sample volume to 200ul by adding buffer DA;

b. If the sample is blood from poultry, birds or amphibians, of which red blood cells have nucleolus, the sample amount should be reduced to 5-20 µl and adjust the volume to 200 µl by adding Buffer DA.

c. Cultured cells: collect about 1x105-106 suspended cells into a 1.5 ml microcentrifuge tube; for adherent cells, trypsinization and pipetting should be used firstly, and then centrifuge at 12,000 rpm for 1 min, discard the supernatant, add 200 µl Buffer DA to the cell pellet, and shake to completely resuspend.

d. Animal tissue: weigh 25 mg (spleen<10 mg). The animal tissue is ground into fine powder in liquid nitrogen or cut into small pieces with a scalpel, and transferred into a 1.5 ml microcentrifuge tube pre-filled with 180 µl Buffer DA.

e. Bacteria: Take 1-5 ml of culture cells, centrifuge at 12,000 rpm for 1 min to collect the bacteria cells, and discard the supernatant as much as possible.

(1) For gram-negative bacteria: add 200 µl Buffer DA to the bacterial pellet, and shake it until the pellet is completely resuspended.

(2) For Gram-positive bacteria: Resuspend in 0.6 ml lysate (preparation of lysate: 30 ml ultrapure water + 600 mg lysozyme (provided by the customer). Divide them into small tubes and keep them at -20°C for long-term storage), mix well by inverting the tubes 5-10 times and incubate at 37°C for at least 30 min (some Gram-positive bacteria may take longer time).

f. Yeast cells:

(1) Take yeast cells (up to 5×107cells), centrifuge at 12,000 rpm for 1 min, and remove the supernatant as much as possible (for more cultured yeast cells solution, collect the yeast cells pellets by several centrifugation into one centrifuge tube).

(2) Destruction of yeast cell wall:

Add 600 µl Sorbitol buffer and about 50 U Lyticase (provided by the customer) to the cells, and mix thoroughly. Incubation at 30°C for 30 min. Centrifuge at 4,000 rpm for 10 min, discard the supernatant, and collect the precipitate.

Note: The Lyticase dosage described as above for 5×107 yeast cells.

According to the yeast strain and the number of yeast cells, the concentration of lyticase and incubation time can be adjusted appropriately.

(3) Add 200 µl Buffer DA to resuspend the pellet and mix well.

2. Optional steps: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) solution (provided by the customer), shake it for 15 sec, and stay at room temperature for 5 min.

3. Add 20 µl Proteinase K, mix well.

a. If the sample is blood or cells, add Proteinase K and mix well, then go to the next step.

b. If the sample is animal tissue, add Proteinase K, mix well by vortex. Incubate it at 56°C until the tissue is completely dissolved. Centrifuge briefly to collect the solution on the inner wall of the cap, and then go to the next step.

Note: For different tissues, it usually takes 1–3 h to finish digestion (rat tail needs to be digested for 6-8 h, if necessary, digestion is done overnight), and it will not affect subsequent operations.

4. Add 200 µl Buffer DLT, mix thoroughly by inverting the tube, incubate at 70°C for 10 min, and centrifuge briefly to collect the solution on the inner wall of the cap.

Note: A white precipitate may be produced when Buffer DLT is added, which will generally disappear when incubated at 70°C and will not affect subsequent experiments.

5. Add 200 µl of absolute ethanol, mix well (precipitation may occur at this point), transfer all the solution with precipitate to the Bind DNA Spin Columns (the adsorption column is placed in the collection tube), and centrifuge at 12,000 rpm for 30 sec, Discard the flow-through, and put the adsorption column back into the collection tube.

6. Add 500 µl Buffer DW1 (please add absolute ethanol before use) to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the flow- through, and put the adsorption column back into the collection tube.

7. Add 500 µl Buffer DW2 (please add absolute ethanol before use) to the adsorption column, centrifuge at 12,000 rpm for 30 sec, discard the flow- through, and put the adsorption column back into the collection tube.

8. Repeat step 7 once.

9. Centrifuge at 12,000 rpm for 3 minutes, discard the flow-through.

10. Transfer the adsorption column to a new 1.5 ml microcentrifuge tube, and add 60-200 µl Buffer EB to the center area of the adsorption membrane. Stay at room temperature for 1 minutes, centrifuge at 12,000 rpm for 1 minutes, and collect the DNA solution in the centrifuge tube.

Note: The volume of Buffer EB should not be less than 60µl, or it will affect the recovery efficiency. If ddH2O is used as eluet, make sure its pH value is between 7.0 and 8.5 (NaOH can be used to adjust the pH value of ddH2O to this range). Genomic DNA should be stored at -20°C to avoid degradation.

[Precautions]

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will get smaller and the DNA yield will decrease.

2. Before the first use, add absolute ethanol to Buffer DW1 and Buffer DW2 indicated on bottle the label.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
	Use By		Date of Manufacture
	Temperature Limitation		Consult Instructions for Use
	Lot Number		Manufacturer
	Number of Tests		Reference Number
			Any warnings and/or precaution to take

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