Universal Column Genome Extraction Kit

Project number: U665923

Storage conditions: room temperature.

Products content:

Component	U66592350 preps	U665923 200preps
BufferGTL	15mL	60mL
BufferGL	15mL	50mL
BufferGW1 (concentrate)	13mL	52mL
BufferGW2 (concentrate)	15mL	70mL
BufferGE	15mL	60mL
ProteinaseK	1.25mL	4×1.25mL
SpinColumnsDMwithCollectionTubes	50	200

Product Introduction:

This kit is suitable for the extraction of high purity total DNA from a wide range of samples such as fresh or frozen animal tissues, cells, blood, bacteria, etc. The product can be purified to obtain DNA fragments with a molecular weight of up to 50kb without the use of toxic solvents such as phenol or chloroform, and without the need for ethanol precipitation. The optimized buffer system enables the DNA in the lysate to bind efficiently and specifically to the silica matrix centrifugal adsorption column, and the inhibitors of PCR and other enzymatic reactions can be removed efficiently through a two-step washing step, and finally eluted with low-salt buffer or water, which results in high-purity DNA. The purified DNA can be directly used for downstream experiments such as enzymatic digestion, PCR, Real-TimePCR, library construction, SouthernBlot and molecular labeling.

Self-contained reagent: anhydrous ethanol

EnzymaticLysisBuffer (required for extraction of genomic DNA from Gram-positive bacteria).

Self-contained reagents: EnzymaticLysisBuffe formulation: 20 mMTris, pH 8.0; 2 mMNa2-EDTA; 1.2%

Triton's own reagent: X-100; Lysozyme (lysozyme) at a final concentration of 20 mg/mL.

Pre-experiment Preparation and Important Notes

1. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller fragments of extracted DNA and a decrease in the amount extracted.

2. If extracting genomes from bacterial cultures with large accumulations of secondary metabolites or thick cell walls, it is recommended that samples be collected early in the logarithmic growth period.

3. Anhydrous ethanol should be added to BufferGW1 and BufferGW2 according to the instructions on the label of the reagent bottle before first use.

4. Before use, please check whether BufferGTL and BufferGL are crystallized or precipitated. If crystallized or precipitated, please re-dissolve BufferGL and BufferGTL in 56℃ water bath.

5. If the downstream experiments are sensitive to RNA contamination, 4 μLDNase-Free RNaseA (100mg/mL) can be added before adding BufferGL, RNaseA is not provided in this kit.

procedure

i Genomic extraction of blood and cell samples

Material handling

1a. If the extracted material is mammalian anticoagulated blood fluid (anucleated red blood cells), add BufferGTL directly to 50-200 μL of fresh or frozen anticoagulated blood fluid sample to make up to 200 μL;

1b. If the extracted material is anticoagulated blood fluid from avian, bird, amphibian, or lower organisms with nucleated erythrocytes, take a 5-10 μL sample of fresh or frozen anticoagulated blood fluid and add BufferGTL to make up to 200 μL;

1c. Cells in adherent culture should be first processed into cell suspension (maximum extraction volume is 5×10^6 cells), centrifuged at 2,000 rpm (400×g) for 5 min, discard the supernatant, add 200 μLGTL, and shake until the sample is thoroughly suspended;

Note: If RNA removal is required, add 4 μL of RNaseA solution at a concentration of 100 mg/mL after the above steps are completed, vortex for 15 seconds, and leave at room temperature for 2 minutes.

2. Add 20 μL of ProteinaseK.

3. Add 200 μLBufferGL, vortex and shake to mix thoroughly, and water bath at 56° C for 10 minutes.

4. Centrifuge briefly to remove water droplets from the inside of the tube cap. Add 200 μL of anhydrous ethanol and mix well by vortexing and shaking. Centrifuge briefly.

Note: 1) Vortex and shake to mix immediately after adding BufferGL and anhydrous

ethanol.

(2) The addition of BufferGL and anhydrous ethanol may produce a white precipitate that will not affect subsequent experiments. Some tissues, upon addition of BufferGL

and anhydrous ethanol may form a sol-gel product, in which case vigorous shaking or vortexing is recommended.

All of the solution obtained in the previous step is added to the adsorption column (SpinColumnsDM) that has been loaded into the collection tube, and if the solution cannot be added all at once, it can be transferred in several times. centrifuge at 12,000 rpm (~ 13,400 × g) for 1 min, pour off the waste solution in the collection tube, and put the adsorption column back into the collection tube.

6. Add 500 μLBufferGW1 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

7. Add 500 μLBufferGW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note: Step 7 can be repeated if further DNA purity is required.

8. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).