Biospin Blood-Cell-Tissue Genomic DNA

BIOER

【Kit Components】

Cat#	BSC47S1	BSC47M1
Components	**50 Tests**	**100 Tests**
TES buffer	15mL	30mL
LysisB buffer	25mL	50mL
PK solution	0.5mL	1mL
PW Buffer	12mL (add 18mL absolute ethanol)	24mL (add 36mL absolute ethanol)
Wash Buffer	24mL (add 36mL absolute ethanol)	48mL (add 72mL absolute ethanol)
Elution Buffer	5mL	10mL
Spin Columns	50	100
Handbook	1	1

【Storage & shipping conditions】

1. Store Protease K at 2~8°C,store all other components at room temperature.
2. All components are stable for 18 months from date of receipt under proper storage condition.

【Introduction】

The kit provides a simple, fast and cost effective m ethod that can isolate high quality DNA. Using one simple protocol, high yield purified DNA can be isolated from v arious samples including animal tissue, whole blood, buffy coat, leukocytes, and cultured cells. The kit utilizes silica -based membrane techniques within a convenient spin column, no need of expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The whole procedure takes less than 30 minutes including lysis steps, and more than 20 kb DNA could be purified by this kit. Isolated DNA can be directly applied to PCR, Southern blotting and other enzymatic reactions.

【Typical genomic DNA yields from various samples】

Source	Quantity	Yield (μg)
Mammalian tissue	25mg	5-30
Mammalian blood	200μL	2-10
Poultry blood / Amphibian	5-20μL	5-40
Cell	$\leq 2\times10^6$	5-30
Blood spots	2-5 pieces	0.1-1

【Additional apparatus and materials required but not supplied】

* Sterile1.5mL/2.0mL microcentrifuge tubes
* 10μL/100μL/1000μL tips
* Centrifuge capable of 12,000g
* Absolute ethanol
* Vortex mixer
* Warm bath
* PBS buffer

【Important notes】

Please add absolute ethanol to PW Buffer and Wash Buffer, mix thoroughly before use.

【Protocol】

➢ **Sample lysis**

■ **Cell and blood**

1 · Sample pre-processing

A. **Culture cells**: adherent cells shall be digested with trypsin, no more than 107cells.Spin for 5min in 1500rpm, discard the supernate. Add 200μL PBS buffer into the tube, re-suspend cells.

B. Leukocytes: if blood volume is more than 200μL, use Red Blood Cell Lysis Buffer (Cat. # BSA06M1 or Cat. # BSA07M1) first to obtain white blood cells, add 200μL PBS buffer into the tube, re-suspend cells.

C. Mammalian Whole blood: Add 200μL well mixed blood into1.5mL tube (if the blood is less than 200μL, add to 200μL with PBS buffer).

D. Birds, amphibians, anticoagulant whole blood： Add 5-20μL well mixed blood into 1.5mL tube · add to 200μL with PBS buffer.

2 · Add 10μL PK solution, mix thoroughly for 15s.

Add 4μL RNase A (Cat. # BSA40S1). Incubate at room temperature for5min.

3 · Add 200μL Lysis B buffer, mix thoroughly. Incubate at 56°C for 10min.

4 · Add 200μL absolute ethanol, mix thoroughly.

■ **Animal tissue**

1 · Add 200μL TES Buffer and 10μL PK solution into tube.

2 · Grind tissue to powder (no more than 25mg) with liquid nitrogen. Add it to the tube and mix thoroughly.

Note: for spleen, no more than 10mg, samples must be submerged within solution.

3 · Incubate for 1-4 hours at 56°C. For samples hard to lysis, prolong lysis time or even overnight.

Note: vortex per 10s will facilitate lysis.

4 · Centrifuge 3min at 12,000g. Carefully transfer the supernatant into a new tube. Add 4μL RNase A (Cat. # BSA40S1). Incubate at room temperature for 5min.

5 · Add 200μL Lysis B buffer, mix thoroughly.

6 · Add 200μL absolute ethanol, mix thoroughly.

■ **Blood spots**

1 · Take 2-5 pieces spots (3×3mm) into a tube.

2 · Add 300μL TES Buffer and 10μL of PK solution into the tube. Mix intensively for 10s. Put it in 56°C thermostat oscillator metal bath, vortex for 60min at 900rpm.

3 · Centrifuge for 3min at 12,000g. Carefully transfer the supernatant into a new tube. Add 4μL RNase A (Cat. # BSA40S1). Incubate at room temperature for 5min.

4 · Add 300μL Lysis B buffer, mix thoroughly.

5 · Add 150μL absolute ethanol, mix thoroughly.

➢ **DNA Purification**

1 · Transfer all the mixture (≤750μL) to spin column. Centrifuge the mixture at 12,000× g for 1 minute. Discard flow-through.

2 · Add 500μL PW buffer to the Spin column. Centrifuge the spin column at 12,000×g for 1 minute. Discard flow-through.

3 · Add 500μL Wash buffer to the spin column. Centrifuge at 12,000 ×g for 1 minute. Discard flow-through. Take the spin column back to the tube.

4 · Repeat step 3

5 · Centrifuge the spin column at 12,000 ×g for 2 minute.

6 · Place the spin column in a new 1.5 or 2.0mL micro centrifuge tube. Add 30-100μL of the Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge the mixture at 10,000×g for 1 minute. The DNA in the collection tube is ready for further analysis. If the isolated DNA sample is not going to be tested on the same day, freeze it at -20°C.