Note: vortex per 10s will facilitate lysis.

4. Centrifuge 3min at 12,000g. Carefully transfer the supernatant into a new tube.

Add $4\mu L$ RNase A (Cat. # BSA40S1). Incubate at room temperature for 5min.

- 5. Add 200 μ L Lysis B buffer, mix thoroughly.
- 6. Add 200µL absolute ethanol, mix thoroughly.

Blood spots

- 1. Take 2-5 pieces spots $(3 \times 3mm)$ into a tube.
- 2. Add 300 μL TES Buffer and 10 μL 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 (\leq 750µL) to spin column. Centrifuge the mixture at 12,000×g for 1 minute. Discard flow-through.

2. Add 500μ L PW buffer to the Spin column. Centrifuge the spin column at $12,000 \times g$ for 1 minute. Discard flow-through.

- 3. Add 500 μ L 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 $12,000 \times g$ for 2 minute.

6. Place the spin column in a new 1.5 or 2.0mL micro centrifuge tube. Add $30-100\mu$ L of the Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge the mixture at $10,000 \times g$ 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.

Company Information

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Biospin Blood/Cell/Tissue Genomic DNA Extraction Kit

TECHNICAL SUPPORT:

For technical support, please dial phone number : 0086-571-87774567-5215 or 5211, or fax to 0086-571-87774553 Email to <u>reagent@bioer.com.cn</u>. Website: www.bioer.com.cn

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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 method that can isolate high quality DNA. Using one simple protocol, high yield purified DNA can be isolated from various 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	$\leq 2 \times 10^{6}$	5-30
Blood spots	2-5 pieces	0.1-1

[Additional apparatus and materials required but not supplied]

- * Sterile1.5mL/2.0mL microcentrifuge tubes
- * 10 μL/100 μL/1000 μL tips * Absolute ethanol
- * Vortex mixer * Warm bath * PBS buffer

[Important notes]

* Centrifuge capable of 12,000g

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 200μ L 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.

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