

Kit Components

Cat#	BSC21S1	BSC21M1
Components	50Tests	100Tests
SP Buffer	45 mL	90 mL
Lysis S Buffer	5 mL	10 mL
DA Buffer	12.5 mL	25 mL
Binding Buffer	35 mL	70 mL
Wash Buffer	30 mL (add 45 mL absolute ethanol before use)	60 mL (add 90 mL absolute ethanol before use)
Elution Buffer	15 mL	30 mL
Grind Tube	50 tubes	100 tubes
Spin Column	50 tubes	100 tubes
Handbook	1 copy	1 copy

Storage

- ◆ All reagents, when stored properly, are stable for 18 months.
- ◆ The kit can be transported at room temperature.

Introduction

The kit provides a very simple, fast and economic way for the isolation of PCR-ready genomic DNA from soil, adopting the Genomic DNA Buffer Set. The simple purification procedure, based on the remarkable selectivity of Biospin membrane, allows isolation of high yields genomic DNA less than 30minutes. It does not require expensive equipment, involves only few steps, and completely avoids the use of toxic and hazardous reagents such as phenol and chloroform.

At first, the soil sample is lysed by SP buffer and Lysis S Buffer in the grind tube. Designed for using with the lysis instruments from BIOER,soil is easily lysed within 40 s.Also manual operation can be chose with vortex generator within 5minutes.

Then DNA in the sample is liberated. After centrifuging, the impurity will be discarded. Released DNA is bound exclusively and specifically to the Biospin membrane in presence of a Binding Buffer under appropriate salt iron and pH conditions. Denatured protein and other contaminants are removed by several washing procedures. The DNA is then eluted from the membrane with the Elution Buffer.

## Apparatus and Materials to Be Supplied by the User

- \* Sterile 2.0mL microcentrifuge tubes      \* 10μL/100μL/1000μL tips
- \* Microcentrifuge capable of 14,000g      \* Absolute ethanol

## Important notes

- Please add 45mL absolute ethanol to Wash Buffer and mix thoroughly before the first use.
- Lysis S Buffer may form precipitates upon storage. In case of precipitate forming, please incubate the buffer at 37°C until the precipitate has fully dissolved.

## Protocol

1. Add 0.5g soil to lysing Grind Tube.
2. Add 900μL SP Buffer and 100μL Lysis S Buffer
3. Homogenize in the lysis instrument for 30s at a speed setting of 5.5m/s. Or vortex for 5 minutes at the maximum speed with vortex generator.
4. Centrifuge at 14,000g for 10 min to pellet debris.
5. Transfer supernatant to a 2.0 microcentrifuge tube.
6. Add 250μL DA Buffer, mix thoroughly.
7. Incubate on ice for 5 minutes, centrifuge at 14,000g for 5 min.
8. Transfer supernatant to a 2.0 microcentrifuge tube.
9. Add 700μL Binding Buffer, mix thoroughly.
10. Transfer the mixture to the Spin Column. Centrifuge at 14,000g for 1 minute. Discard flow-through. Sample volume is more than 700μL, simply load and spin again.
11. Add 600μL Washing Buffer to the Spin Column. Centrifuge at 14,000g for 1 minute. Discard flow-through.
12. Repeat step 11th.
13. Centrifuge for an additional 2 minute at 14,000g and transfer the Spin Column to a sterile 1.5mL microcentrifuge tube.
14. Add 100μL to 200μL Elution Buffer, Incubate at room temperature for 1 minute.
15. Centrifuge at 14,000g for 1 minute. The buffer in the microcentrifuge tube contains the DNA. The purified DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20°C if not used immediately.

## FAQ

Q1 : The optimal extraction quantity to extract soil samples ?

A : The optimum extraction quantity of soil is 0.5 g . For different wet soil samples, 0.25 g to 0.5 g of soil samples is suggested.

Q2 : If DNA does not amplify ?

A :An excess amount of DNA will inhibit a PCR reaction. Gradient diluting the template DNA to choose the optimum concentration.

Q3 : Alternative Lysis Methods ?

A : If cells are difficult to lyse, vortexing 10 minutes after adding SP Buffer and lysis S buffer.

Q4 : How to concentrating the DNA?

A : .The final volume of eluted DNA will be 100μl .The DNA may be concentrated by adding 4μl of 5M NaCl and votexing 5 times to mix. Add 200μl of 100% cold ethanol and invert 5 times to mix. Centrifuge at 12000×g for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac,dessicator,or air dry.Resuspend precipitated DNA in sterile water or sterile 10mM Tris.

Q5 : How to guarantee the complete genome DNA ?

A : Intense shock force and centrifugal force will inevitably result genomic DNA be sheared . So if you want to get complete genome DNA, you can properly reduce the amplitude of grinding machine. Also we can reduce the centrifugal force from 14000g to 10000g.