Technical Bulletin

# GenElute™ Soil DNA Isolation Kit

**DNB100**

## Product Description

GenElute™ Soil DNA Isolation Kit provides a convenient and rapid method for the detection of microorganisms from soil samples. A simple and rapid spin column procedure is then used to purify the DNA. Total genomic DNA can be isolated and purified from all the various microorganisms found in soil, such as bacteria, fungi, and algae.

Purification is based on spin column chromatography. The process involves first adding the soil sample, Lysis Buffer G, and Lysis Additive A to a provided Bead Tube, and the soil sample is homogenized. The sample is then centrifuged, and the supernatant is transferred to a DNase-free microcentrifuge tube. Binding Buffer I is added, and the lysate is incubated for 5 minutes on ice. To remove particles, the lysate is then centrifuged and transferred to a new DNAase-free tube. Solution OSR is added, tubes are mixed by inversion, incubated for 5 minutes on ice, and again centrifuged to remove any particles. The cleared supernatant is transferred to a fresh DNAase-free tube and Lysis Buffer QP is added along with ethanol; the sample is then vortexed. Next, the solution is loaded onto a spin-column, which binds only the DNA. The bound DNA is then washed using the provided Binding Buffer B and Wash Solution A, and the purified DNA is eluted using the Elution Buffer B and is now ready for use.

## Kit Components

| Materials Provided | 50 preps |
|---|---|
| Lysis Buffer G | 45 mL |
| Lysis Additive A | 2 x 6 mL |
| Binding Buffer I | 7 mL |
| OSR Solution | 3 mL |
| Lysis Buffer QP | 25 mL |
| Binding Buffer B | 30 mL |
| Wash Solution A | 18 mL |
| Elution Buffer B | 8 mL |
| Bead Tubes | 50 |
| Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
| Product Insert | 1 |

## Reagents and Equipment Required But Not Provided

- Benchtop microcentrifuge
- 1.7 mL DNase-free microcentrifuge tubes
- DNAse-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 96-100% ethanol

## Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Reagents to be prepared

Before beginning the procedure, prepare the following:

- Prepare a working concentration of Wash Solution A by adding 42 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Spin Columns from the GenElute™ Soil DNA Isolation Kit (white contents with a plastic o-ring).

## Storage/Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

## Procedures

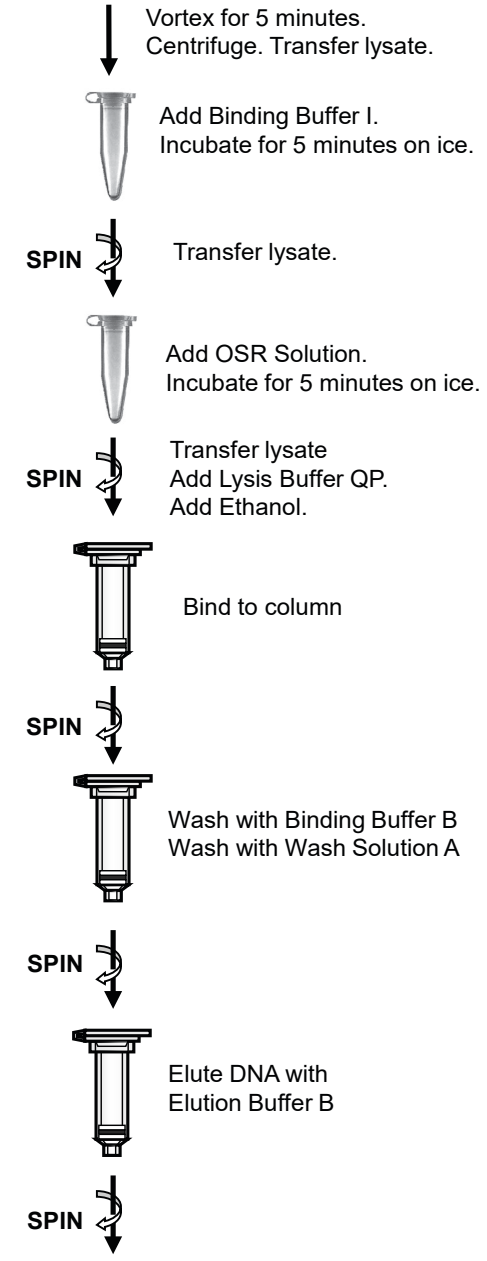
**Note:** All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check the microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force.

## Purifying Total DNA using GenElute™ Soil DNA Isolation Kit Flowchart

Add soil sample, Lysis Buffer G and Lysis Additive A to Bead Tube.



**Ensure that all solutions are at room temperature prior to use.**

## Lysate Preparation

1. Add 250 mg of soil sample (maximum input varies depending on the sample type) to a provided Bead Tube and add 750 µL of Lysis Buffer G briefly to mix soil and Lysis Buffer G.

   **Note:** In the case of a wet soil sample, transfer the sample to a clean 1.7 mL microcentrifuge tube and centrifuge for 30 seconds at

   20,000 x g (~14,000 rpm). Remove the water carefully using a pipette, and resuspend the soil pellet in 750 µL of Lysis Buffer G. Transfer the soil to a Bead Tube using a pipette. Proceed to Step 1b.
2. Add 200 µL of Lysis Additive A and vortex briefly.
3. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g., FastPrep®-24 Instrument). Vortex for 30 seconds at 4 M/S using a FastPrep®-24 instrument, or 5 minutes using a flat-bed vortexer at maximum speed. Alternatively, optimize the time and speed according to the manufacturer's manual.
4. Centrifuge the tube for 2 minutes at 20,000 x g (~14,000 rpm).
5. Transfer clean supernatant to a DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
6. Add 100 µL of Binding Buffer I, mix by inverting the tube a few times, and incubate for 5 minutes on ice.
7. Spin the lysate for 2 minutes at 20,000 x g (~14,000 rpm) to pellet any protein and soil particles.
8. Using a pipette, transfer up to 700 µL of supernatant into a DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
9. Add 50 µL of OSR Solution, mix by inverting the tube a few times, and incubate for 5 minutes on ice. Spin the lysate for 2 minutes at 20,000 x g (~14,000 rpm) to pellet any protein and soil particles.
10. Using a pipette, transfer up to 700 µL of clean supernatant to a DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
11. Add 400 µL of Lysis Buffer QP and 550 µL of 96-100% ethanol (provided by the user). Vortex briefly. Proceed to next step.

## Binding to Column

1. Assemble a provided Spin Column (o-ring) with one of the provided collection tubes.
2. Gently mix the lysate, Lysis Buffer QP, and ethanol using a pipette and apply 600 µL of the clarified lysate onto the column and centrifuge for 30 seconds at 10,000 X g (~10,000 rpm). Discard the flowthrough and reassemble the spin column with the collection tube.
3. Repeat step above with the remaining lysate.

   **Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

## Column Wash

1. Apply 500 µL of Binding Buffer B to the column and centrifuge for 1 minute at 10,000 x g (~10,000 rpm).

   **Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
2. Discard the flowthrough and reassemble the spin column with its collection tube.
3. Apply 500 µL of Wash Solution A to the column and centrifuge for 1 minute at 10,000 x g (~10,000 rpm).
4. Discard the flowthrough and reassemble the spin column with its collection tube.
5. Repeat steps 3 and 4 above.
6. f. Spin the column for 2 minutes at 20,000 x g (~14,000 rpm) in order to thoroughly dry the resin. Discard the collection tube.

## DNA Elution

1. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
2. Add 100 µL of Elution Buffer B to the column and incubate for 1 minute at room temperature.
3. Centrifuge for 1 minute at 10,000 x g (~10,000 rpm).

   **Optional:** An additional elution may be performed if desired by repeating steps 4b and 4c using 50 µL of Elution Buffer in a different elution tube. The total yield can be improved by an additional 20-30% when this second elution is performed.

## Storage of DNA

The purified genomic DNA can be stored at 2-8 °C for a few days.
For longer term storage, –20 °C is recommended.

# Troubleshooting

| Problem | Possible Cause | Solution and Explanation |
|---|---|---|
| Poor DNA Recovery | Homogenization was incomplete | Depending on the type of soil, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 10 minutes at maximum speed. |
| | Lysis Additive A was not added to the lysate | Ensure that the provided Lysis Additive A is added to separate humic acid and increase DNA yield. |
| | 96-100% Ethanol was not added to the lysate | Ensure that 230 µL of 96-100% ethanol is added to the lysate before binding to the column. |
| | Ethanol was not added to the Wash Solution A | Ensure that 42 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use. |
| DNA does not perform well in downstream applications | Eluted DNA sample is brown | The elution contains a high concentration of humic acids. Ensure that the OSR Solution was added to the clean lysate. |
| | DNA was not washed with the provided Lysis Buffer QP and Wash Solution A | Traces of humic acids or salt from the binding step may remain in the sample if the column is not washed with the provided Lysis Buffer QP and Wash Solution A. Humic acids and salt may interfere with downstream applications, and thus must be washed from the column. |
| | Ethanol carryover | Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |
| | PCR reaction conditions need to be optimized | Take steps to optimize the PCR conditions being used, including varying the amount of template (10 ng to 20 ng for 20 µL of PCR reaction is recommended), changing the source of Taq polymerase, looking into the primer design and adjusting the annealing conditions. |

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