
Version: 011818

FastBeat Soil DNA Kit (Bead Beating)

FastBeat 土壤 DNA 提取试剂盒 (珠磨法)

Cat#: DN45

❖ Kit Contents and Storage

Kit Contents	Storage	50 Preps (DN4501)
Bead Tube	RT	50
Sodium Phosphate Buffer	RT	50 ml
MT Buffer	RT	6 ml
PPS Solution	RT	13 ml
IRS Solution	RT	15 ml
PQ Solution	RT	35 ml <i>Add indicated ethanol before first use</i>
Buffer WB	RT	13 ml <i>Add indicated ethanol before first use</i>
Elution Buffer	RT	15 ml
DNA Bind Columns	RT	50

All reagents, when store in indicated temperature, are stable for 9 months.

❖ Description

The FastBeat Soil DNA Kit quickly and efficiently isolates PCR-ready genomic DNA directly from soil samples in less than 40 minutes. Designed for use with Beads-Beating device such as the FastPrep® Instruments from MP Biomedicals, soil organisms population are easily lysed within 40 seconds. Samples are placed into 2.0 ml tubes containing 3 kinds of beads, a mixture of ceramic and silica particles designed to efficiently lyse all soil organisms including historically difficult sources such as eubacterial spores and endospores, gram positive bacteria, yeast, algae, nematodes and fungi.

The kit uses a novel and proprietary method to remove high humic acid content including difficult soil types such as compost, sediment, and manure. The isolated DNA has a high level of purity allowing for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g. *Bacillus subtilis*, *Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and Actinomycetes (e.g. *Streptomyces*).

❖ **Important consideration before use**

The fill volume in the bead tube after the addition of the Sodium Phosphate and MT Buffers to the sample should allow sufficient air space in the sample tube for efficient FastPrep® Instrument processing. MP Biomedicals recommends using 500 mg of starting material as long as there is between 250 - 500 µl of empty space in the tube. Sample loss or tube failure may result from overfilling the bead tube. The bead tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes. The kits have been rigorously tested in the FastPrep® Instrument. A single 40 second run at a speed setting of 6.0 in the FastPrep® Instrument is sufficient to lyse almost all samples. If the user experimentally determines that additional processing time is required, the sample should be incubated on ice in the bead tube for at least 2 minutes between successive FastPrep® Instrument homogenizations to prevent overheating the sample and tube.

If you use other bead beater device, please follow instruction manual from manufacturer to set appropriate parameter for good performance.

❖ Procedure

Note:

⇒ Before the first use, add the indicated amount of ethanol into PQ solution bottle, Buffer WB bottle, mix well, and mark the bottle with a check.

1. Add up to 500 mg of soil sample to a Bead Tube.
2. Add 980 µl Sodium Phosphate Buffer to sample in Bead Tube. Gentle vortex to mix. Add 120 µl MT Buffer.

Note: Check MT Buffer. If MT Buffer is precipitated, heat solution to 60°C until dissolved before use.

3. Homogenize in the FastPrep® Instrument for 40 seconds at a speed setting of 6.0.
4. Centrifuge at 12,000 x g for 5 minutes to pellet debris.
5. Transfer supernatant to a clean 2.0 ml centrifuge tube. Add 250 µl PPS Solution and mix by shaking the tube by hand 10 times. Incubate at 4°C for 5 minutes.
6. Centrifuge tubes at 10,000 x g for 3 minutes at room temperature. Avoiding pellet, transfer up to, but no more than, 900 µl of supernatant to a clean 2 ml centrifuge tube.
7. Add 300 µl of IRS Solution (1/3 volume) and vortex briefly. Incubate at 4°C for 5 minutes.
8. Centrifuge tubes at 10,000 x g for 1 minute at room temperature. Avoiding pellet, transfer the supernatant into a clean 5 ml centrifuge tube.
9. Add 1.5 volumes of PQ Solution to the cleared supernatant and mix by pipetting.

Example: To 1100 µl lysate add 1650 µl PQ Solution. Reduce the amount of PQ Solution accordingly if less supernatant is recovered. A precipitate may form after the addition of ethanol but this will not affect the procedure.

Note: Ensure ethanol has been added to PQ Solution.

Note: It is important to pipet PQ Solution directly onto the cleared supernatant and to mix immediately.

10. Load approximately 700 μl mixture onto Spin Filter(sitting in collection tube) and centrifuge at 10,000 x g for 1 minute at room temperature. Discard flow through. Load another 700 μl and repeat until all remaining mixture is loaded on Spin Filter.

Note: A total of 4-5 loads for each sample processed may be required.

11. Add 600 μl of Buffer WB to Spin Filter and centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow through. Repeat Step 11 with another 600 μl Buffer WB.

Note: Ensure ethanol is added to Buffer WB.

12. Centrifuge Spin Filter at 13,000 x g for 2 minute at room temperature to dry the Spin Filter..

13. Carefully place Spin Filter in clean 1.5 ml centrifuge tube. Avoid splashing any Buffer WB onto Spin Filter. Add 100 μl of Elution Buffer (Optional: pre-warm the water to 70–90°C will increase the DNA yield) to the center of the column membrane. Incubate at room temperature for 3-5 min, and centrifuge at 13,000 x g for 1 min to elute the DNA.

Note: Use smaller volume(minimum 30 μl) of Elution Buffer will obtain higher concentration.

Optional: Put eluate back to the Spin Column to repeat elution once. This increases concentration of DNA about 10-15%.