AipBest 土壤基因组 DNA 提取试剂盒（珠磨法）

AipBest Soil Genome DNA Extraction Kit（Bead Beating）

使 用 说 明 书

Version：011818

◆Cat#：OD203

◆Kit Contents and Storage

Kit Contents	Storage	50 Preps (OD203-01)
Bead Tube	RT	50
Sodium Phosphate Buffer	RT	50mL
MT Buffer	RT	6mL
PPS Solution	RT	13mL
IRS Solution	RT	15mL
PQ Solution	RT	35mL *Add indicated ethanol before first use*
Buffer WB	RT	13mL *Add indicated ethanol before first use*
Elution Buffer	RT	15mL
DNA Bind Columns	RT	50

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

◆Description：

The AipBest Soil Genome DNA Extraction Kit quickly and efficiently isolates PCR-ready genomic DNAdirectly 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 2.0mL 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 - 500µL 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 manufaturer 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 980µL Sodium Phosphate Buffer to sample in Bead Tube. Gentle votex to mix. Add 120µL 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 12,000xg for 5minutes to pellet debris.
5. Transfer supernatant to a clean 2.0mL centrifuge tube. Add 250µL PPS Solution and mix by shaking the tube by hand 10 times. Incubate at 4°C for 5 minutes.
6. Centrifuge tubes at 10,000xg for 3 minute at room temperature. Avoiding pellet, transfer up to, but no more than, 900µL of supernatant to a clean 2mL centrifuge tube.
7. Add 300µL of IRS Solution(1/3 volume) and vortex briefly. Incubate at 4°C for 5 minutes.
8. Centrifuge tubes at 10,000xg for 1 minute at room temperature. Avoiding pellet, transfer the supernatant into a clean 5mL centrifuge tube.
9. Add 1.5 volumes of PQ Solution to the cleared supernatant and mix by pipetting.
   Example: To 1100µL lysate add 1650µL 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 700µL mixture onto Spin Filter(sitting in collection tube) and centrifuge at 10,000xg for 1 minute at room temperature. Discard flow through. Load another 700µL and repeat untill all remaining mixture is loaded on Spin Filter.
    Note: A total of 4-5 loads for each sample processed may be required.
11. Add 600µL of Buffer WB to Spin Filter and centrifuge at 10,000xg for 30 seconds at room temperature. Discard flow through. Repeat Step 11 with another 600µL Buffer WB.
    Note: Ensure ethanol is added to Buffer WB.
12. Centrifuge Spin Filter at 13,000xg for 2 minute at room temperature to dry the Spin Filter..
13. Carefully place Spin Filter in clean 1.5mL centrifuge tube. Avoid splashing any Buffer WB onto Spin Filter. Add 100µL 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 13,000xg for 1 min to elute the DNA.
    Note: Use smaller volume(minimum 30µL) 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%.

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