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FastBeat Soil DNA Pro Kit (Bead Beating)

Product Number: DNK4801

Shipping and Storage

All reagents, when store in indicated temperature, are stable for 12 months.

Component

Component	DNK4801-50T
Bead Tube	50
Balance Solution	5mL
Sodium Phosphate Buffer	50mL
MT Buffer	6mL
SR Solution	15mL
GH Solution	15mL
	Add Isopropanol before first use
Buffer WB	13mL
	Add ethanol before first use
Elution Buffer	10mL
DNA Bind Columns	50

Description

The FastBeat Soil DNA Pro 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 2.0mL 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.

Application

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 500mg 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.

Protocol

Before the first use, add isopropanol according to the label on the GH solution bottle and anhydrous ethanol according to the label on the Buffer WB bottle. Mix well and mark the label to indicate that ethanol or isopropanol has been added.

1. Add a maximum of 500 mg of soil sample to the grinding tube. .
2. Add 980μL Sodium Phosphate Buffer and 120μL MT Buffer to the soil sample.
   Check MT Buffer before use. If the cold weather causes precipitation in MT Buffer, it can be re dissolved by heating at 60°C, gently reversing and mixing before use.
3. Use a FastPrep sample preparation apparatus at 6.0 m/s, grind for 40 seconds, or vortex for 10 minutes.
   The above grinding conditions are suitable for most samples, but the required grinding speed and time for individual samples need to be determined after experimentation. If FastPrep instrument is not available, a vortex shaker (recommended with corresponding adapter) can be used to vortex at maximum speed for 10 minutes to lyse the sample.
4. Centrifuge at 13000 rpm for 5-10 minutes to precipitate impurities.
5. Carefully transfer the supernatant (approximately 800μL) to a new 2mL centrifuge tube (provided). Add 300μL SR Solution, vortex thoroughly for 10 seconds, centrifuge at 13000 rpm for 1 minute. Transfer the supernatant to a 2mL centrifuge tube (be careful not to suck up any possible sediment at the bottom).
6. Column equilibrium: Add 100μL of equilibrium solution to the adsorption column AC, centrifuge at 13000 rpm for 1 minute, discard the filtrate, and set aside for later use.
   The equilibrium solution can enhance the adsorption capacity of silica gel membrane for nucleic acids. Please use the adsorption column treated on the same day.
7. Add an equal volume of GH Solution to the supernatant, vortex or blow to mix well.
   Ensure that the specified amount of isopropanol has been added to the GH Solution bottle before use.
   Ensure that GH Solution is directly added to the supernatant and mixed immediately.
8. Add 720μL of the previous mixture (including possible precipitates) into an adsorption column AC (place the adsorption column in a collection tube), centrifuge at 13000 rpm for 30 seconds, and discard the waste liquid from the collection tube. Place the centrifuge column in the recovery manifold and repeat until all the mixture is added.
   It takes about 3 repetitions to transfer all the mixture onto the centrifuge column.
9. Add 600μL Buffer WB (please check if anhydrous ethanol has been added first!)Centrifuge at 13000 rpm for 30 seconds and discard the waste liquid. Add 600μL of Buffer WB and rinse again.
10. Return the adsorption column AC to the empty collection tube, centrifuge at 13000 rpm for 2 minutes, and try to remove the rinsing solution as much as possible to prevent residual ethanol in the rinsing solution from inhibiting downstream reactions.
11. Take out the adsorption column AC and place it in a clean 1.5 ml centrifuge tube. Add 50-80μL Elution Buffer (the elution buffer is preheated in a 80-90°C water bath for better effect) to the middle of the adsorption membrane. Let it stand at room temperature for 3-5 minutes and centrifuge at 13000 rpm for 1 minute.
    The larger the elution volume, the higher the elution efficiency. If a higher DNA concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μL. If the volume is too small, it will reduce the elution efficiency and DNA yield.
    Add the obtained eluent back into the centrifugal adsorption column, let it stand at room temperature for 2 minutes, and centrifuge at 13000 rpm for 1 minute. Repeated elution can increase the concentration by about 10-15%.
12. The eluted DNA can be used immediately or stored at -20°C.