CYDN48-CHANGYU FastBeat Soil DNA Pro Kit (Bead Beating)

Catalog number	Package
CYDN4801	50 preps
CYDN4802	100 preps

Storage and shipping: all components are shipped at ambient temperature; stable for 12 months when stored at the indicated temperature after receipt. All solutions should be clear; if a precipitate forms at low ambient temperature, warm in a 37 °C water bath for a few minutes until clear, mixing by gentle inversion and avoiding excessive foaming. Keep all solutions tightly capped after use to avoid evaporation, oxidation and pH drift.

Applications

For extraction from soil and environmental samples including lawn soil, flower bed soil, flower pot soil, farmland soil, forest soil, sludge, water samples, red soil, black soil and dust.

Kit components

Component	Storage	50 preps (CYDN4801)	100 preps (CYDN4802)
Bead tubes	15-25 °C	50	100
Balance Solution	15-25 °C	5 ml	10 ml
Lysis Buffer SPB	15-25 °C	50 ml	100 ml
Buffer MT	15-25 °C	6 ml	12 ml
Buffer SR	15-25 °C	15 ml	30 ml
Buffer GH	15-25 °C	15 ml (add isopropanol before first use)	30 ml (add isopropanol before first use)
Wash Buffer WB	15-25 °C	13 ml (add ethanol before first use)	25 ml (add ethanol before first use)
Elution Buffer EB	15-25 °C	10 ml	10 ml
Spin columns AC (with collection tubes)	15-25 °C	50	100

Before first use, add isopropanol to Buffer GH and absolute ethanol to Wash Buffer WB as indicated on the bottle labels, mix thoroughly and tick the boxes on the labels.

Description

The FastBeat Soil DNA Pro Kit is an upgraded version of the CYDN45 FastBeat Soil DNA Kit. It rapidly and efficiently isolates PCR-ready genomic DNA from soil samples in less than 40 minutes. The kit is designed for use with bead-beating devices (for example the FastPrep® series instruments from MP Biomedicals); soil microorganisms are lysed within 40 seconds. Samples are added to 2 ml centrifuge

tubes preloaded with three kinds of beads, a mixture of ceramic and silica particles designed to efficiently lyse all soil microorganisms, including targets that are otherwise difficult to lyse: eubacterial spores, endospores, Gram-positive bacteria, yeasts, algae, nematodes and fungi.

The kit uses a dedicated process to remove the high concentrations of humic acid present in samples and is suitable for difficult soil matrices such as compost, sediment and manure. The extracted DNA has high purity and markedly improves the success rate of PCR amplification from soil microorganisms. The kit has been used 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). DNA recovered with this kit carries little inhibitory contamination and is of good integrity; it can be used directly for PCR, restriction digestion, high-throughput sequencing, metagenomic microbial diversity analysis and other downstream experiments.

Features

Maximizes yield and purity and improves the success of downstream applications including PCR amplification and high-throughput sequencing.

Bead-beating lysis with preloaded ceramic/silica beads efficiently lyses difficult soil microorganisms, including eubacterial spores, endospores, Gram-positive bacteria, yeasts, algae, nematodes and fungi.

Notes before starting

Note: A bead beater (or a vortex mixer as a substitute), ethanol and isopropanol must be supplied by the user.

Note: After adding Lysis Buffer SPB and Buffer MT to the sample in the bead tube, sufficient air space must remain above the liquid to ensure efficient FastPrep® homogenization. MP Biomedicals recommends a starting sample amount of 500 mg with 250-500 µl of empty space remaining in the tube. Overfilling the bead tube may cause sample loss or even tube breakage. The bead tube cap must be tightly sealed but not over-tightened, to prevent sample leakage. If a single tube cannot hold the whole sample, divide the sample among several tubes.

Note: The kit has been rigorously validated in the FastPrep® instrument: a single 40-second run at a speed setting of 6.0 lyses the vast majority of soil samples. If experiments show that some samples require a longer disruption time, place the bead tube on ice for at least 2 minutes between successive FastPrep® homogenizations to avoid overheating and damage to the sample and tube from continuous high-speed friction.

Note: If another brand of bead-beating tissue homogenizer is used, set the parameters according to the manufacturer's instructions to ensure good extraction. Users without a homogenizer can substitute vortexing for 10 minutes.

❖ Procedure

Before first use, add isopropanol to Buffer GH and absolute ethanol to Wash Buffer WB as indicated on the labels, mix thoroughly and tick the boxes on the labels.

1. Add no more than 500 mg of soil sample to the bead tube.

2. Add 980 µl of Buffer SPB and 120 µl of Buffer MT to the soil sample.

Check Buffer MT before use. If a precipitate forms in cold weather, redissolve by heating at 60 °C, mix by gentle inversion and use.

3. Process in a FastPrep sample preparation instrument at 6.0 m/s for 40 seconds, or vortex for 10 minutes.

The above disruption conditions suit most samples; for individual samples the disruption speed and time may need to be determined experimentally. If no FastPrep instrument is available, a vortex mixer (preferably with the appropriate

adapter) can be used at maximum speed for 10 minutes to lyse the sample.

4. Centrifuge at 13,000 rpm for 5-10 minutes to pellet impurities.

5. Carefully transfer the supernatant (about 800 µl) to a new 2 ml centrifuge tube (supplied by the user). Add 300 µl of Buffer SR, vortex for 10 seconds to mix thoroughly and centrifuge at 13,000 rpm for 1 minute. Transfer the supernatant to a 2 ml centrifuge tube, taking care not to take up any precipitate at the bottom.

6. Column equilibration: add 100 µl of Balance Solution to spin column AC, centrifuge at 13,000 rpm for 1 minute and discard the flow-through; the column is now ready.

The Balance Solution enhances the nucleic acid binding capacity of the silica membrane; use columns equilibrated on the same day.

7. Add an equal volume of Buffer GH to the supernatant and mix by vortexing or pipetting.

Make sure that the indicated amount of isopropanol has been added to the Buffer GH bottle before use.

Make sure that Buffer GH is added directly to the supernatant and mixed immediately.

8. Load 720 µl of the mixture from the previous step (including any precipitate) onto spin column AC placed in a collection tube; centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Return the column to the collection tube and repeat until the whole mixture has been loaded.

About 3 loads are usually needed to pass the whole mixture through the column.

9. Add 600 µl of Buffer WB (check first that absolute ethanol has been added), centrifuge at 13,000 rpm for 30 seconds and discard the flow-through. Add another 600 µl of Buffer WB and repeat the wash once.

10. Return column AC to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

11. Transfer column AC to a clean 1.5 ml centrifuge tube. Apply 50-80 µl of Elution Buffer to the centre of the membrane (prewarming the buffer in an 80-90 °C water bath improves the result), leave at room temperature for 3-5 minutes and centrifuge at 13,000 rpm for 1 minute.

A larger elution volume gives higher elution efficiency. If a higher DNA concentration is required, the elution volume may be reduced, but not below 30 µl; too small a volume reduces elution efficiency and DNA yield.

Reload the eluate onto the column, leave at room temperature for 2 minutes and centrifuge at 13,000 rpm for 1 minute. A second elution increases the concentration by about 10-15%.

12. The eluted DNA can be used immediately or stored at -20 °C.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.