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 *Add indicated ethanol before first use*
Buffer WB	RT	13 ml *Add indicated ethanol before first use*
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 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 2.0 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 manufaturer to set appropriate parameter for good performance.

❖ 操作步骤

注意：

⇨ **第一次使用前，按照PQ solution 瓶子和Buffer WB 瓶子上的标签指示加入指定量无水乙醇，加入乙醇后混匀，在标签上打钩表明已经加入了乙醇。**

1. 向研磨管中加入最多不超过500 mg 土壤样本。.
2. 向土壤样本中加入 980 µl Sodium Phosphate Buffer, 120µl MT Buffer。

   注意：用前检查 MT Buffer。 如果天气冷导致 MT Buffer 出现沉淀，可以 60°C 加热重新溶解，轻柔颠倒混匀后使用。
3. 使用 FastPrep 样品制备仪，6.0 m/s，研磨 40 sec，或涡旋震荡 10 min。

   备注：以上研磨条件适合于大多数样本，但个别样本需要的研磨速度和时间需要尝试后再确定。如果没有 FastPrep 仪器，可使用涡旋震荡仪（推荐搭配相应适配器），以最大速度涡旋 10 min 来裂解样本。
4. 14, 000 x g 离心 5 -10 min 沉淀杂质。
5. 小心地将上清液（约 800 µl）转移至新的 2 ml 离心管（自备）。加入 250 µl PPS Solution ，用手上下剧烈振摇 10 次混匀。.
6. 14, 000 x g 离心 5 min 。转移上清液（约 900 µl）到 2 ml 离心管（注意不要吸到底部可能的沉淀）。
7. 加入 300 µl IRS Solution（三分之一体积），短暂涡旋混匀， 4°C 放置 5 min。
8. 14, 000 x g 离心 1 min。 转移上清液到 5 ml 离心管（注意不要吸到底部可能的沉淀）。
9. 加入 1.5 倍体积的 PQ Solution 到上清液中，吹打混匀。

   举例：在 1100 µl 上清液中加入 1650 µl PQ Solution。 如果上清液不足 1100 µl，可以按照比例减少 PQ Solution 用量。加入乙醇后可能有沉淀，这不会影响实验结果。

   注意：使用前确保 PQ Solution 瓶子中已经加入了指定量乙醇。

   注意：确保 PQ Solution 直接加入上清液并立刻吹打混匀。
10. 将上一步混合物 720 µl（包括可能有的沉淀）加入一个吸附柱 AC 中，（吸附柱放入收集管中），14, 000 x g 离心 30 sec，倒掉收集管中的废液。

将离心柱放回收集管，重复直到所有的混合物都加完。

注意：大约需要重复 4 次才能将所有的混合物都上到离心柱上.

11. 加入 600 µl Buffer WB（请先检查是否已加入无水乙醇!），14, 000 x g 离心 30 sec，弃掉废液。再加入 600 µl Buffer WB 重复漂洗一遍。
12. 将吸附柱 AC 放回空收集管中，14, 000 x g 离心 2 min，尽量除去漂洗液，以免漂洗液中残留乙醇抑制下游反应。.
13. 取出吸附柱 AC，放入一个干净的 1.5 ml 离心管中，在吸附膜的中间部位加 100 µl Elution Buffer（洗脱缓冲液事先在 70 - 90°C 水浴中预热效果更好）， 室温放置 3 - 5 min，14, 000 x g 离心 1 min。

    注意： 洗脱体积越大，洗脱效率越高，如需要 DNA 浓度较高，可以适当减少洗脱体积， 但是最小体积不应少于 30 µl，体积过小降低 DNA 洗脱效率，减少 DNA 产量。

    可选：将得到的洗脱液重新加入离心吸附柱中，室温放置 2 min，14, 000 x g 离心 1 min。重复洗脱一次可以提高浓度约 10-15%。
14. 洗脱下来的 DNA 可以立刻使用，或者可以放置在- 20°C 保存。