# MBead Bacteria Genomic DNA Kit

Cat No. PDM03-0100(S)
Size: 100 Reactions

## Description

The MBead Bacteria Genomic DNA Kit provides a fast, simple, and cost-effective method for isolating the genomic DNA from bacterial cells. Its unique buffer system will efficiently lyse cells and degrade protein, allowing for DNA to be easily bound by the surface of the magnetic beads. The Phenol extraction and ethanol precipitation are not required, and the high-quality genomic DNA is eluted in the Release Buffer. The genomic DNA purified with the MBead Bacteria Genomic DNA Kit is suitable for a variety of routine applications. The entire procedure can be completed within 15-20 minutes (using most magnetic separators) or the kit can be easily adapted to satisfy most automated Nucleic Acid purification systems.

## Specifications

- Sample: Up to 300 µl of bacteria
- Operation time: Within 15 minutes (manual)
- Applications: Restriction Enzyme Digestion, Southern Blotting, PCR and qPCR assays
- Storage: Room temperature

## Kit Contents

| Contents | PDM03-0100 | PDM03-0100S |
|---|---|---|
| Magnetic Bead | 2 ml | 80 µl |
| Lysis Buffer | 30 ml | 1.5 ml |
| Wash Buffer | 80 ml | 4 ml |
| Release Buffer | 20 ml | 1 ml |

## Required Materials

- Absolute EtOH
- Magnetic separator
- 1.5 ml microcentrifuge tubes
- Water bath / Dry bath

## Protocol

### Step 1 Lysis

1. Transfer up to 300 µl of the bacterial culture into a 1.5 ml microcentrifuge tube and add 300 µl of Lysis Buffer.
2. Mix well and incubate at 65°C for 5 minutes. During this time, pre-heat the Release Buffer to 65ºC for Step 4.
3. Add 300 µl of absolute EtOH to the lysate and mix well.

### Step 2 DNA Binding

1. Add 20 µl of Magnetic Beads. Mix well by gently shaking for 3 minutes.
2. Place the tube in a magnetic separator for 30 seconds.
3. Remove the solution (if the mixture becomes viscous, increase magnetic bead separation time).

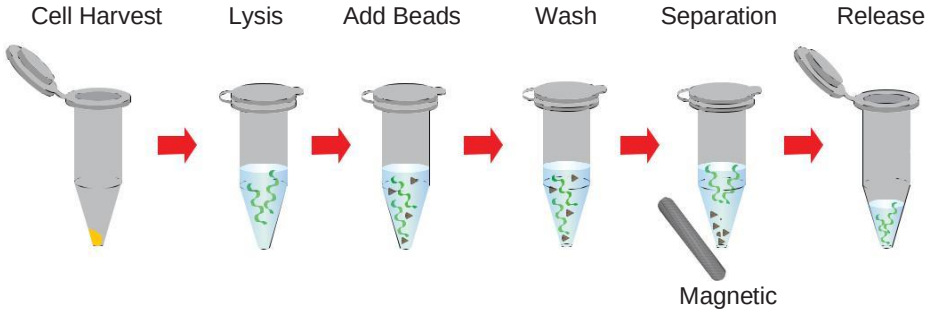
### Step 3 Wash

1. Add 800 µl of the Wash Buffer and mix well (following the wash, the mixture will no longer be viscous).
2. Place the tube in a magnetic separator for 30 seconds. Remove the solution.

### Step 4 Release

1. Add 200 µl of the Release Buffer (pre-heated to 65ºC) and mix well.
2. Incubate for 3 minutes at 65ºC (during incubation, shake the tube vigorously every minute).
3. Place the tube in a magnetic separator for 1 minute.
4. Carefully transfer ONLY the clean portion of the solution to a clean tube.

NOTE: Be sure and allow Magnetic Beads to disperse completely during the binding, wash and elution steps.



## Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying genomic DNA with the kit.

| Problem | Cause | Solution |
|---|---|---|
| DNA is sheared or degraded | Lysate mixed too vigorously | Use the appropriate pipette tip set for the required volume, lower it under the reading line of the solution to mix the sample, pipet up and down gently to mix. |
| | Water used in elution step | Check that the water pH is above 7.0 to avoid the acidic hydrolysis of the elute over time. |
| | DNase contaminated | Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents). |
| RNA containment | Incomplete removal of the RNase. | RNase A treatment |
| Low yields of gDNA | Incomplete lysis and homogenization | Reduce the amount of the starting material. |
| | Incorrect handling of Magnetic Beads | Vortex the tube containing the Magnetic Beads to fully resuspend the beads before adding them to your sample. |
| | Incorrect elution conditions | Repeat magnetic separation and transfer the eluate to a clean tube |
| | The quality of the starting material may not be optimal. | Use the fresh sample and process immediately after collection, or freeze the sample at -80°C or in the liquid nitrogen. |
| High background on UV measurement | Residual beads released | Repeat magnetic separation and transfer the eluate to a clean tube. |

## Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.