Magnetic Beads gDNA Kit (Bacteria)

For research use only

Catalogue Numbers	Quantity
ME048	48 rxns
ME096	96 rxns

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Magnetic Beads Genomic DNA Extraction Kit Bacteria was designed specifically for efficient genomic DNA purification from Gram (-) negative and Gram (+) positve bacteria. DNA is bound to the surface of the magnetic beads and released using a proprietary buffer system. The kit can be easily adapted to automated magnetic bead separation instruments and workstations. The purified DNA can be used in qPCR and a variety of other downstream applications.

Quality Control

The quality of the The Magnetic Beads Genomic DNA Extraction Kit Bacteria is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system by isolating genomic DNA from 1 x 10^9 cells of *Escherichia coli* and from 1 x 10^9 cells of *Bacillus subtilis*. 5 µl of extracted DNA product from a 100 µl eluate was analyzed on a 0.8% agarose gel.

Advantages

- High Yield, High Quality DNA: >26 µg of Genomic DNA, A260/A280 = 1.8-2.0
- Easily adapted to automated magnetic bead separation instruments and workstations
- Sample: 2 x 10^9 bacterial cells
- Operation time: within 30 minutes (manual)

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Components and Storage

Item	Volume	Product	Shipping	Storage
ME1 Buffer	1.5 ml	ME004	room temperature	dry at room temperature (15-25ºC)
	20 ml	ME048		
	30 ml	ME096		
ME2 Buffer	2 ml	ME004	room temperature	dry at room temperature (15-25ºC)
	15 ml	ME048		
	30 ml	ME096		
ME3 Buffer	2 ml	ME004	room temperature	dry at room temperature (15-25ºC)
	15 ml	ME048		
	30 ml	ME096		
ME4 Buffer[1] (Add Isopropanol)	0.5 ml (2 ml)	ME004	room temperature	dry at room temperature (15-25ºC)
	5 ml (20 ml)	ME048		
	10 ml (40 ml)	ME096		
Lysozyme[2]	8 mg	ME004	room temperature	dry at -20ºC for extended periods
	50 mg	ME048		
	110 mg	ME096		
Proteinase K[3] (Add ddH_2O)	1 mg (0.1 ml)	ME004	room temperature	dry at 4ºC
	11 mg (1.1 ml)	ME048		
	11 mg x 2 (1.1 ml x 2)	ME096		
ME Magnetic Beads	220 µl	ME004	room temperature	dry at room temperature (15-25ºC)
	2.5 ml	ME048		
	5 ml	ME096		
MW1 Buffer	2 ml x 2	ME004	room temperature	dry at room temperature (15-25ºC)
	45 ml	ME048		
	60 ml	ME096		
MW2 Buffer[4] (Add Ethanol)	1 ml (4 ml)	ME004	room temperature	dry at room temperature (15-25ºC)
	12.5 ml (50 ml)	ME048		
	25 ml (100 ml)	ME096		
Elution Buffer	1 ml	ME004	room temperature	dry at room temperature (15-25ºC)
	10 ml	ME048		
	30 ml	ME096		

[1]Add Isopropanol (see the bottle label for volume) to ME4 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid Isopropanol evaporation.

[2]Lysozyme is shipped room temperature and should be stored at -20ºC for extended periods after receiving the kit.

[3]Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC.

[4]Add absolute ethanol (see the bottle label for volume) to MW2 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Magnetic Beads Genomic DNA Extraction Kit Bacteria Protocol

IMPORTANT BEFORE USE:

1. Vortex magnetic beads to ensure they are in suspension prior to initial use.
2. Be sure and allow magnetic beads to disperse completely during the binding, wash and elution steps.
3. Add Isopropanol (see the bottle label for volume) to ME4 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid Isopropanol evaporation.
4. Add ddH_2O to Proteinase K (see the bottle label for volume) then vortex to ensure Proteinase K is completely dissolved. Check the box on the bottle. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. For extended periods, the ddH_2O and Proteinase K mixture should be stored at 4ºC.
5. Add absolute ethanol (see the bottle label for volume) to MW2 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional requirements: absolute ethanol, microcentrifuge tubes, 15 ml centrifuge tube (Gram positive only), magnetic separator, isopropanol

Gram (-) Negative Bacteria

Transfer bacteria cells (up to 2 x 10^9) to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. **Add 180 µl of ME1 Buffer** then re-suspend the cell pellet by vortex or pipette. **Add 20 µl of Proteinase K (make sure ddH_2O was added)**. Mix by vortex then incubate at 60ºC for at least 10 minutes. During incubation, invert the tube every 3 minutes. Proceed with step 2 Cell Lysis.

Gram (+) Positive Bacteria

Transfer bacteria cells (up to 2 x 10^9) to a 1.5 ml microcentrifuge tube. Centrifuge for 1 minute at 14-16,000 x g then discard the supernatant. **Transfer the required volume of ME2 Buffer (180 µl/sample)** to a 15 ml centrifuge tube. **Add Lysozyme (0.8 mg/200 µl)** to the **ME2 Buffer** in the 15 ml centrifuge tube then vortex to completely dissolve the **Lysozyme**. **Transfer 180 µl of ME2 Buffer (make sure Lysozyme was added)** to the sample in the 1.5 ml microcentrifuge tube then re-suspend the cell pellet by vortex or pipette. Incubate at 37ºC for 30 minutes. During incubation, invert the tube every 10 minutes. **Add 20 µl of Proteinase K (make sure ddH_2O was added)**. Mix by vortex then incubate at 60ºC for at least 10 minutes. During incubation, invert the tube every 3 minutes. Proceed with step 2 cell lysis.

2. **Add 200 µl of ME3 Buffer** to the sample then mix by vortex for 10 seconds. Incubate the sample at 70ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, invert the tube every 3 minutes.

Optional Step: RNA Degradation (if RNA-free genomic DNA is required)
Following 70ºC incubation, add 5 µl of RNase A (50 mg/ml) to the clear lysate then mix by shaking vigorously and incubate at room temperature for 5 minutes.

3. **Add 500 µl of ME4 Buffer (make sure isopropanol was added)** and mix well by vortex. Vortex **ME Magnetic Beads** for 10 seconds to ensure they are kept in suspension before use. **Add 50 µl of ME Magnetic Beads** then shake gently for 5 minutes to ensure the **ME Magnetic Beads** disperse completely. Place the tube in a magnetic separator for 30 seconds or until **ME Magnetic Beads** have pelleted. Remove and discard the supernatant.

4. Add 600 µl of MW1 Buffer and gently shake the tube for 1 minute. Place the tube in a magnetic separator for 30 seconds or until **ME Magnetic Beads** have pelleted. Remove and discard the supernatant. **Add 600 µl of MW2 Buffer (make sure ethanol was added)** and gently shake the tube for 1 minute. Place the tube in a magnetic separator for 30 seconds or until **ME Magnetic Beads** have pelleted. Remove and discard the supernatant. **Add 600 µl of MW2 Buffer (make sure ethanol was added)** and gently shake the tube for 1 minute. Place the tube in a magnetic separator for 30 seconds or until **ME Magnetic Beads** have pelleted. Remove and discard the supernatant.

5. Incubate the tube at 65ºC for 3 minutes to dry the **ME Magnetic Beads**. **Add 50–200 µl of Elution Buffer**. Mix the sample by pipetting then incubate at room temperature for 3 minutes. During incubation, keep the **ME Magnetic Beads** in suspension by mixing. Place the tube in a magnetic separator for 30 seconds or until **ME Magnetic Beads** have pelleted. Carefully transfer the supernatant containing the purified DNA to a clean 1.5 ml microcentrifuge tube.