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Introduction

The E-Z 96 MagSi Bacterial DNA Kit allows rapid and reliable isolation of high-quality genomic DNA (gDNA) from a wide variety of bacterial species. Up to 0.5 mL gram-positive or gram-negative bacterial culture can be processed each time. The key to the system is Omega Bio-tek's proprietary MagSi® Particle that reversibly binds DNA or RNA under optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

NOTE: MagSi Bacterial DNA Kit will isolate all cellular DNA, including plasmid DNA.

Overview

If using the E-Z 96 MagSi Bacterial DNA Kit for the first time, please read this booklet to become familiar with the procedures. After bacterial cells are collected from culture or picked from an agar plate, the bacterial cell wall is removed by two digestion steps; first with lysozyme and second with Proteinase K. Following lysis, binding conditions are adjusted and the sample is mixed with MagSi Particles to bind the DNA. Three rapid wash steps remove trace salts and protein contaminants and DNA is eluted in water or low ionic strength buffer. Purified DNA can be used directly in downstream applications without the need for further purification.

Storage and Stability

All components of the E-Z 96 MagSi Bacterial DNA Kit, except the Proteinase K, RNase A, and Lysozyme, can be stored at 22-25°C and are guaranteed for at least 12 months from the date of purchase. Once reconstituted in water, lysozyme must be stored at -20°C. Proteinase K should be stored at 15-25°C. Store RNase A at -20°C. Under cool ambient conditions, a precipitate may form in the MSL Buffer or MB2 Buffer. In case of such an event, heat the bottle at 37°C to dissolve.

Kit Contents

Product	M2350-00	M2350-01	M2350-02
Purification	1 x 96	4 x 96	20 x 96
Mag-Si® Particles	1.1 mL	4.2 mL	21 mL
MB1	25 mL	100 mL	2 x 250 mL
MB2	3 mL	12 mL	60 mL
MSL	25 mL	100 mL	2 x 250 mL
SPM Wash Buffer	36 mL	2x72 mL	10 x 72 mL
Elution Buffer	25 mL	100 mL	2 x 250 mL
Lysozyme	120 mg	480 mg	2.4 g
Proteinase K	2 x 1.2 mL	9.6 mL	48 mL
RNase A	550 µL	2.2 mL	5 x 2.2 mL
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Before Starting

Important

Prepare a lysozyme stock solution at 50 mg/mL and aliquot into adequate portions. **Store each aliquot at -20°C and thaw before use**. Each sample will require 18 µL of this solution.

M2350-00 Dissolve with 2.4 mL of Elution Buffer
M2350-01 Dissolve with 9.6 mL of Elution Buffer
M2350-02 Dissolve with 48 mL of Elution Buffer

Dilute **SPM Wash Buffer** with ethanol as follows:

M2350-00 Add 84 mL ethanol (96%-100%)
M2350-01 Add 168 mL ethanol (96%-100%) to each bottle.
M2350-02 Add 168 mL ethanol (96%-100%)to each bottle.

Carry out all centrifugation step at room temperature.

E-Z 96 MagSi Bacterial DNA Protocol from Culture or Agar Plates

Materials to Be Provided by User

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 mL Round bottom plate
- Vortex
- Optional: Lysostaphin (1 mg/mL)
- Ethanol (96%-100%) - Do not use other alcohols
- Multi-channel Pipettor and tips
- Water bath or heating block set at 37°C, 60°C.

Before Starting

- Prepare SPM Buffer according to Preparing Reagents Section on Page 4.
- Set an incubator to 37°C
- Set an incubator to 60 °C

1. Preparing Cells
   a. Cells from Culture:
   Pellet cells from no more than 0.5 mL bacterial culture in a round bottom processing plate (1.2 mL) by centrifugation at 4,000 x *g* for 10 minutes at room temperature. Carefully aspirate the media without disturbing the cell pellet and discard. Proceed to Step 2.

   b. Cells from an Agar Plate
   If you pick a colony from agar plate, add 90 µL of MB1 Buffer and 10 µL of Lysozyme (50 mg/mL) into each well of the sample plate. Add a colony into a well and mix thoroughly. Proceed to Step 3.

2. Add 90µL MB1 Buffer and by adding 10µL lysozyme (50 mg/mL) to the sample. Mix the sample thoroughly by pipetting or vortexing. Make sure the cells are fully resuspended.
   Note: For some species of staphylococci, add 1-2 µL lysostaphin (1 mg/mL). Lysostaphin is not supplied.

3. Incubate at 37°C for 10 minutes. Vortex the plate 1-2 times during incubation.

   Note: The amount of enzyme required and/or the incubation time may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time might yield better results.

4. Add 12 µL MB2 Buffer and 20 µL Proteinase K solution and mix throughly by pipetting.

5. For gram-negative bacteria, incubate at 60°C in a shaking water bath for 20 minutes. For gram-positive bacteria, incubate at 60°C in a shaking water bath for 40-60 minutes. Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate and shake plate every 20-30 minutes.

6. Add 5 µL RNase A to each sample and mix the samples by pipetting up and down 5 times. Incubate at room temperature for 5 minutes.

7. Add 135 µL MSL Buffer and 10 µL MagSi Particles E and mix the sample thoroughly by vortexing.

8. Add 182 µL ethanol (96-100%) and mix thoroughly by pipetting up and down 20 times.

9. Incubate at room temperature for 5 minutes.

10. Place the plate on a magnetic separation device to collect the magnetic particles. The solution should be clear after all the particles are pelleted on the corner of each well adjacent to the magnet.

11. Without removing the plate from the magnet, carefully aspirate and discard the cleared supernatant without disturbing the magnetic beads pellet.

12. Remove the plate from the magnetic separation device. Add 480 µL SPM

buffer to the each sample and resuspend the magnetic particles by pipetting up and down 20 times.

13. Place the tube on a magnetic separation device to collect the magnetic particles.
14. Without removing the plate from the magnet, carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.

15. Remove the plate from the magnetic separation device. Add 480 µL of SPM buffer to each sample and resuspend the magnetic particles by pipetting up and down 20 times.

16. Place the plate on a magnetic separation device to collect the magnetic beads.

17. Remove and discard the cleared supernatant. Leave the plate on the magnetic separation device for 5 minutes to dry the magnetic particles. Remove any residual liquid with a pipettor.

18. Remove the plate from the magnetic separation device, add 200 µL Elution Buffer. Resuspend the particles by vortexing for 30 seconds. Incubate at 60°C for 5-10 minutes.

19. Place the plate in a magnetic separation device to collect the magnetic particles. Transfer the cleared supernatant containing the eluted DNA into a new 96-well microplate.

Isolating Bacterial DNA from Viscous or Mucous Samples

Materials to Be Provided by User

- Centrifuge with rotor and adaptor for microplate
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 mL Round bottom plate
- Vortex
- Optional: Lysostaphin (1 mg/mL)
- Ethanol (96%-100%) - Do not use other alcohols
- Multiple Channel Pipettor and tips
- Waterbath or heating block set at 37°C, 60°C.

Before Starting

- Prepare SPM Buffer According to Preparing Reagents Section on Page 4.
- Set an Incubator to 37°C
- Set an Incubator to 60°C

1. Add 200 µL of sample into a 96-well deep-well plate (1.2 or 2 mL).

2. Dilute the sample with 200 µL MB1 Buffer with freshly prepared DTT solution to the final concentration of 0.15% (w/v). Incubate at 37°C until the sample can be pipetted.

3. Transfer 200 µL of sample into a new deep-well plate.

4. Add 20 µL lysozyme (20 mg/mL) into the sample and incubate at 37°C for 10 minutes.

5. Add 25 µL MB2 Buffer and 20 µL Proteinase K solution. Mix the samples thoroughly by vortexing.

6. For gram-negative bacteria, incubate at 55°C in a shaking water bath for 20 minutes. For gram-positive bacterial, incubate at 60°C in a shaking water bath for 40-60 minutes. Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate and vortex the samples every 20-30 minutes.

7. Add 5 µL RNase A to samples and mix the sample thoroughly by vortexing the plate. Incubate at room temperature for 5 minutes.

8. Add 245 µL MSL Buffer and 10 µL MagSi Particles E and mix the sample thoroughly by vortexing.

9. Add 330 µL ethanol (room temperature, 96-100%) and mix thoroughly by vortexing for 20 seconds.
10. Transfer half of the sample volume into a round-bottom 96-well plate.

Place the plate onto a magnetic separation device. Wait until all the magnetic particles are cleared from solution.

11. Carefully aspirate and discard the supernatant.

12. Repeat Steps 10-11 until all of the magnetic particles from samples are collected.

13. Remove the plate from the magnetic separation device. Add 480 µL SPM buffer to the each sample and resuspend the magnetic particles by pipetting up and down 20 times.

14. Place the tube on a magnetic separation device to collect the magnetic particles.

15. Without removing the plate from the magnet, carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.

16. Remove the plate from the magnetic separation device. Add 480 µL of SPM buffer to each sample and resuspend the magnetic particles by pipetting up and down 20 times.

17. Place the plate on a magnetic separation device to collect the magnetic particles.

18. Remove and discard the cleared supernatant. Leave the plate on the magnetic separation device for 5 minutes to dry the magnetic particles. Remove any residual liquid with a pipettor.

19. Remove the plate from the magnetic separation device, add 200 µL Elution Buffer. Resuspend the particles by vortexing for 30 seconds. Incubate at 60°C for 5-10 minutes.

20. Place the plate in a magnetic separation device to collect the magnetic particles. Transfer the cleared supernatant containing the eluted DNA into a new 96-well microplate.

Isolating Bacterial DNA from Urine

Materials to Be Provided by User

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 mL round-bottom plate
- Vortex
- Optional: Lysostaphin (1 mg/mL)
- Ethanol (96%-100%) - Do not use other alcohols
- Multi-channel Pipettor and tips
- Water bath or heating block set at 37°C, 60°C.

Before Starting

- Prepare SPM Buffer According to Preparing Reagents Section on Page 4.
- Set an Incubator to 37°C
- Set an Incubator to 60 °C

1. Add 1 mL of urine sample into a 96-well deep-well plate.

2. Centrifuge at 3000 x *g* for 5 minutes.

3. Discard the supernatant and add 200µl MB1 Buffer. Resuspend the bacterial cell pellet by vortexing for 20 seconds.

4. Add 20 µL lysozyme (20 mg/ml) into the sample and incubate at 37°C for 10 minutes.

5. Add 25 µL MB2 Buffer and 20 µL Proteinase K solution. Mix the sample thoroughly by vortexing.

6. For gram-negative bacteria, incubate at 55°C in a shaking water bath for 20 minutes. For gram-positive bacteria, incubate at 60°C in a shaking water bath for 40-60 minutes. Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate and vortex the samples every 20-30 minutes.

7. Add 5 µL RNase A to each sample and mix thoroughly by vortexing the plate. Incubate at room temperature for 5 minutes.

8. Add 245 µL MSL Buffer and 10 µL MagSi Particles E. Mix the samples thoroughly by vortexing.

9. Add 330 µL ethanol (room temperature, 96-100%) and mix thoroughly by vortexing for 20 seconds.

10. Transfer half of the sample volume into a round-bottom 96-well plate. Place the plate onto a magnetic separation device. Wait until all the

magnetic particles are cleared from solution.

11. Carefully aspirate and discard the supernatant.

12. Repeat Steps 10-11 until all of the magnetic particles from samples are collected.

13. Remove the plate from the magnetic separation device. Add 480 µL SPM buffer to the each sample and resuspend the magnetic particles by pipetting up and down 20 times.

14. Place the tube on a magnetic separation device to collect the magnetic particles.

15. Without removing the plate from the magnet, carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.

16. Remove the plate from the magnetic separation device. Add 480 µL of SPM buffer to each sample and resuspend the magnetic particles by pipetting up and down 20 times.

17. Place the plate on a magnetic separation device to collect the magnetic beads.

18. Remove and discard the cleared supernatant. Leave the plate on the magnetic separation device for 5 minutes to dry the magnetic particles. Remove any residual liquid with a pipettor.

19. Remove the plate from the magnetic separation device, add 200 µL Elution Buffer. Resuspend the particles by vortexing for 30 seconds. Incubate at 60°C for 5-10 minutes.

20. Place the plate in a magnetic separation device to collect the magnetic particles. Transfer the cleared supernatant containing the eluted DNA into a new 96-well microplate.

Isolating Bacterial DNA from Body Fluids

Materials to Be Provided by User

- Centrifuge with rotor and adaptor for microplates
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 mL round-bottom plate
- Vortex
- Optional: Lysostaphin (1 mg/mL)
- Ethanol (96%-100%) - Do not use other alcohols
- Multi-channel Pipettor and tips
- Water bath or heating block set at 37°C, 60°C.

Before Starting

- Prepare SPM Buffer According to Preparing Reagents Section on Page 4.
- Set an Incubator to 37°C
- Set an Incubator to 60 °C

1. Add 100 µL sample into a 96-well deep-well plate.

2. Add 100 µL MB1 Buffer. Mix the samples thoroughly by vortexing for 20 seconds.

3. Add 20 µL lysozyme (20 mg/ml) to each sample and incubate at 37°C for 10 minutes.

4. Add 25 µL MB2 Buffer and 20 µL Proteinase K to each sample. Mix the samples thoroughly by vortexing.

5. For gram-negative bacteria, incubate at 55°C in a shaking water bath for 20 minutes. For gram-positive bacteria, incubate at 60°C in a shaking water bath for 40-60 minutes. Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate and shake or vortex the samples every 20-30 minutes.

6. Add 5 µL RNase A to each sample and mix thoroughly by vortexing. Incubate at room temperature for 5 minutes.

7. Add 245 µL MSL Buffer and 10 µL MagSi Particle E and mix the samples thoroughly by vortexing.

8. Add 330 µL ethanol (room temperature, 96-100%) and mix thoroughly by vortexing for 20 seconds.

9. Transfer half of the sample volume into a round-bottom 96-well plate. Place the plate onto a magnetic separation device. Wait until all the magnetic particles are cleared from solution.

10. Carefully aspirate and discard the supernatant.

11. Repeat Steps 10-11 until all of the magnetic particles from samples are collected.

12. Remove the plate from the magnetic separation device. Add 480 µL SPM buffer to the each sample and resuspend the magnetic particles by pipetting up and down 20 times.

13. Place the tube on a magnetic separation device to collect the magnetic particles.

14. Without removing the plate from the magnet, carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.

15. Remove the plate from the magnetic separation device. Add 480 µL of SPM buffer to each sample and resuspend the magnetic particles by pipetting up and down 20 times.

16. Place the plate on a magnetic separation device to collect the magnetic beads.

17. Remove and discard the cleared supernatant. Leave the plate on the magnetic separation device for 5 minutes to dry the magnetic particles. Remove any residual liquid with a pipettor.

18. Remove the plate from the magnetic separation device, add 200 µL Elution Buffer. Resuspend the particles by vortexing for 30 seconds. Incubate at 60°C for 5-10 minutes.

19. Place the plate in a magnetic separation device to collect the magnetic particles. Transfer the cleared supernatant containing the eluted DNA into a new 96-well microplate.

Isolating Bacterial DNA from Secretion Swabs (buccal swab or nasal swab)

Materials to Be Provided by User

- Centrifuge with rotor and adaptor for microplate
- Magnetic Separation Device (Cat# MSD-01)
- 1.2 mL Round bottom plate
- Vortex
- Optional: Lysostaphin (1 mg/mL)
- Ethanol (96%-100%) - Do not use other alcohols
- Multi-channel Pipettor and tips
- Water bath or heating block set at 37°C, 60°C.

Before Starting

- Prepare SPM Buffer According to Preparing Reagents Section on Page 4.
- Set an Incubator to 37°C
- Set an Incubator to 60 °C

1. Submerge the swab tip into a deep-well plate containing 280 µL MB1 Buffer in each well.

2. Add 20 µL lysozyme (20 mg/ml) to each sample and incubate at 37°C for 10-15 minutes.

3. Transfer 200 µL sample into a new deep well plate.

4. Add 20 µL MB2 Buffer and 20 µL Proteinase K to each sample. Mix the samples thoroughly by vortexing.

5. For gram-negative bacteria, incubate at 55°C in a shaking water bath for 20 minutes. For gram-positive bacteria, incubate at 60°C in a shaking water bath for 40-60 minutes. Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate and shake or vortex the samples every 20-30 minutes.

6. Add 5 µL RNase A to each sample and mix thoroughly by vortexing. Incubate at room temperature for 5 minutes.

7. Add 245 µL MSL Buffer and 10 µL MagSi Particle E and mix the samples thoroughly by vortexing.

8. Add 330 µL ethanol (room temperature, 96-100%) and mix thoroughly by vortexing for 20 seconds.

9. Transfer half of the sample volume into a round-bottom 96-well plate. Place the plate onto a magnetic separation device. Wait until all the

magnetic particles are cleared from solution.

10. Carefully aspirate and discard the supernatant.

11. Repeat Steps 10-11 until all of the magnetic particles from samples are collected.

12. Remove the plate from the magnetic separation device. Add 480 µL SPM buffer to the each sample and resuspend the magnetic particles by pipetting up and down 20 times.

13. Place the tube on a magnetic separation device to collect the magnetic particles.

14. Without removing the plate from the magnet, carefully aspirate and discard the cleared supernatant without disturbing the magnetic particles.

15. Remove the plate from the magnetic separation device. Add 480 µL of SPM buffer to each sample and resuspend the magnetic particles by pipetting up and down 20 times.

16. Place the plate on a magnetic separation device to collect the magnetic beads.

17. Remove and discard the cleared supernatant. Leave the plate on the magnetic separation device for 5 minutes to dry the magnetic particles. Remove any residual liquid with a pipettor.

18. Remove the plate from the magnetic separation device, add 200 µL Elution Buffer. Resuspend the particles by vortexing for 30 seconds. Incubate at 60°C for 5-10 minutes.

19. Place the plate in a magnetic separation device to collect the magnetic particles. Transfer the cleared supernatant containing the eluted DNA into a new 96-well microplate.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	Inefficient cell lysis	• Increase the lysozyme incubation time • Increase the Proteinase K digestion time
	SPM Buffer is not prepared correctly	Prepare the SPM, Buffer by adding ethanol according to instruction
	Lose of magnetic beads during operation	Do not aspirate the magnetic particles during the operation
	Inefficient cell lysis due to decrease of activity of proteinase k	Add more Proteinase K solution.
No DNA eluted.	SPM Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPM Wash Buffer Concentrate as instructed on the label.
Problem with downstream application	Insufficient DNA was used	1. Use more starting material 2. Quantify the purified DNA accurately and use sufficient DNA.
	Excess DNA was used for downstream application	Make sure to use correct amount DNA.