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Introduction

The E-Z 96 Mag-bind 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. Key to the system is Omega Bio-Tek's proprietary Mag-Bind® Particle that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

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NOTE: Mag-Bind Bacterial DNA Kit will isolate all cellular DNA, including plasmid DNA.

Overview

If using theE-Z 96 Mag-Bind 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 agar plate, the bacterial cell wall is removed by lysozyme digestion, followed by Proteinase K digestion. Following lysis, binding conditions are adjusted and the sample is mixed with Mag-Bind particles to bind DNA. Three rapid wash steps remove trace salts and protein contaminants, and finally DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E-Z 96 Mag-Bind Bacterial DNA Kit, except the Proteinase K, RNase A and Lysozyme can be stored at 22°C-25°C and are guaranteed for at least 12 months from the date of purchase. Once reconstituted in water, Proteinase K and lysozyme must be stored at -20°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-Bind Particles E	1.1 ml	4.2 ml	21 ml
MB1	25 ml	100 ml	500 ml
MB2	3 ml	12 ml	60 ml
MSL	25 ml	100 ml	500 ml
SPM Wash Buffer	30 ml	150 ml	2 x 300 ml
Elution Buffer	25 ml	100 ml	500 ml
Lysozyme	120 mg	480 mg	2.4 g
Proteinase K	50 mg	200 mg	1 g
Proteinase Storage Buffer	3 ml	11 ml	55 ml
RNase A	30 µl	275 µl	1.1 ml
User Manual	1	1	1

Materials to Be Provided by User

- Centrifuge with rotor and adaptor for microplate
- Magnetic Separation Device (Cat# MSD-01)
- 500 ul Round bottom process plate (EZ9604)
- Absolute ethanol (96%-100%) - Do not use other alcohols
- Multiple Channel Pipettor and tips
- Waterbath or heating block set at 37°C, 60°C or 75°C.

Before Starting

Please read the entire booklet to become familiar with the Mag-Bind Bacterial DNA Kit procedure.

Important

Prepare a stock solution of Proteinase K (provided) as follows and aliquot into adequate portions. **Store aliquots at -20°C.**

M2350-00 Dissolve with 2.5 ml of Proteinase Storage Buffer
M2350-01 Dissolve with 10 ml of Proteinase Storage Buffer
M2350-02 Dissolve with 50 ml of Proteinase Storage Buffer

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 70 ml absolute (96%-100%) ethanol
M2350-01 Add 350 ml absolute (96%-100%) ethanol
M2350-02 Add 800 ml absolute (96%-100%) ethanol

Carry out all of centrifugation step at room temperature.

E-Z 96 Mag-Bind Bacterial DNA Protocol

The following protocol is designed for isolating genomic DNA from up to 0.5 ml culture.

1. Harvest no more than 0.5 ml bacterial culture in a round bottom processing plate (500µl) by centrifugation at 4,000 x g for 10 min at room temperature. If you pick up colony from agar plate, place the colony into plate and mix throughly with 90 µl MB 1 Buffer as described in step2.

2. Resuspend the cell pellet with 90µl MB1 buffer and by adding 10µl Lysozyme (50mg/m) to the sample. Mix the sample throughly by pipetting or vortexing. Make sure the cell are fully resuspended.

   Note: for some species of staphylococci, add 1-2µl 1mg/ml lysostaphin (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 bacterial, incubate at 60°C for 20 minutes. For gram positive bacterial, incubate at 75°C for 40-60 minutes. Usually no more than 1 h is required for bacterial lysis. If no shaking waterbath is available, incubate and shake plate every 20-30 minutes.

6. Add 5 µl RNase A to samples and mix the sample by pipetting. Incubate at room temperature for 5 minutes.

7. Add 135 µl Buffer MSL and 10µl Mag-Bind Particle E and mix the sample throughly by shaking or brief vortexing.

8. Add 182 µl absolute ethanol (room temperature, 96-100%) and mix thoroughly by pipetting up and down for 20 times.

9. Incubate at room temperature for 5 minutes.

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

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

12. Remove the plate from the magnetic separation device. Add 400 µl of SPM buffer to the plate and resuspend the magnetic beads by pipetting up and down for 20 times.

13. Place the tube in a magnetic separation device to collect the magnetic beads.

14. Without remove the tube from the magnet, carefully remove and discard the cleared supernatant without disturbing the magnetic beads pellet.

15. Remove the plate from the magnetic separation device. Add 400 µl of SPM buffer to the plate and resuspend the magnetic beads by pipetting up and down for 20 times.

16. Place the tube in 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 beads. Remove any drop of the liquid with pipettor.

18. Remove the tube from the magnetic separation device, add 200µl Elution Buffer. Resuspend the beads by vortexing for 30 seconds. For high DNA yield, incubate at 60°C for 5-10 minutes .

19. Place the tube in a magnetic separation device (MSD-02) to collect the magnetic beads. Transfer the cleared supernatant contains eluted DNA into a new 1.5 ml tube.

Isolating bacterial DNA from viscous or mucous sample

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

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

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

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

5. Add 25 µl MB2 Buffer and 20µl Proteinase K solution. Mix the sample throughly by brief vortexing.

6. For gram negative bacterial, incubate at 55°C for 20 minutes. For gram positive bacterial, incubate at 75°C for 40-60 minutes. Usually no more than 1 h is required for bacterial lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.

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

8. Add 245 µl Buffer MSL and 10µl Mag-Bind Particle E and mix the sample throughly by shaking or brief vortexing.

9. Add 330 µl absolute ethanol (room temperature, 96-100%) and mix thoroughly by vortexing at maxi speed for 20 seconds.

10. Transfer half volume of sample (415 µl) into a round bottom well process plate (500µl). And place the plate onto a magnetic separation device. Wait until all the magnetic beads are cleared from solution.

11. Remove and discard the supernatant. Transfer remaining sample (410µl) from step 9 into the plate. Repeat step 10-11 to collect magnetic beads.

12. Proceed the washing and elution by following step 12-19 in the standard protocol on page 6-7.

Isolating bacterial DNA from Urine

1. Add 1 ml of urine sample into a 96 deep well plate.
2. Centrifuge at 3000 x g for 5 minutes.
3. Transfer 200µl sample into a new deep-well plate
4. Discard the supernatant and add 200µl MB1 Buffer. Resuspend the bacterial cell pellet by vortexing for 20 seconds.
5. Add 20µl lysozyme (20mg/ml) into the sample and incubate at 37°C for 10 minutes.
6. Add 25 µl MB2 Buffer and 20µl Proteinase K solution. Mix the sample throughly by brief vortexing.
7. For gram negative bacterial, incubate at 55°C for 20 minutes. For gram positive bacterial, incubate at 75°C for 40-60 minutes. Usually no more than 1 h is required for bacterial lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.
8. Add 5 µl RNase A to samples and mix the sample throughly by vortexing the plate. Incubate at room temperature for 5 minutes.
9. Add 245 µl Buffer MSL and 10µl Mag-Bind Particle E and mix the sample throughly by shaking or brief vortexing.
10. Add 330 µl absolute ethanol (room temperature, 96-100%) and mix thoroughly by vortexing at maxi speed for 20 seconds.
11. Transfer half volume of sample (415 µl) into a round bottom well process plate (500µl). And place the plate onto a magnetic separation device. Wait until all the magnetic beads are cleared from solution.
12. Remove and discard the supernatant. Transfer remaining sample (410µl) from step 9 into the plate. Repeat step 10-11 to collect magnetic beads.
13. Proceed the washing and elution by following step 12-19 in the standard protocol on page 6-7.

Isolating bacterial DNA from body fluids

1. Add 100 µl sample into a 96 deep well plate.
2. Add 100 µl MB1 Buffer. Mix the sample throughly by vortexing for 20 seconds.
3. Add 20 µl lysozyme (20mg/ml) into the sample and incubate at 37°C for 10 minutes.
4. Add 25 µl MB2 Buffer and 20µl Proteinase K solution. Mix the sample throughly by brief vortexing.
5. For gram negative bacterial, incubate at 55°C for 20 minutes. For gram positive bacterial, incubate at 75°C for 40-60 minutes. Usually no more than 1 h is required for bacterial lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.
6. Add 5 µl RNase A to samples and mix the sample throughly by vortexing the plate. Incubate at room temperature for 5 minutes.
7. Add 245 µl Buffer MSL and 10µl Mag-Bind Particle E and mix the sample throughly by shaking or brief vortexing.
8. Add 330 µl absolute ethanol (room temperature, 96-100%) and mix thoroughly by vortexing at maxi speed for 20 seconds.
9. Transfer half volume of sample (415 µl) into a round bottom well process plate (500µl). And place the plate onto a magnetic separation device. Wait until all the magnetic beads are cleared from solution.
10. Remove and discard the supernatant. Transfer remaining sample (410µl) from step 9 into the plate. Repeat step 10-11 to collect magnetic beads.
11. Proceed the washing and elution by following step 12-19 in the standard protocol on page 6-7.

Isolating bacterial DNA from secretion swabs (buccal swab or nasal swab)

1. Submerge the swab tip into a deep well plate contains 280µl MB1 Buffer in each well.

2. Add 20µl lysozyme (20mg/ml) into the 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 20µl Proteinase K solution. Mix the sample throughly by brief vortexing.

5. For gram negative bacterial, incubate at 55°C for 15 minutes. For gram positive bacterial, incubate at 65°C for 30 minutes. Usually no more than 1 h is required for bacterial lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.

6. Add 5 µl RNase A to samples and invert tube several times to mix. Incubate at room temperature for 5 minutes.

7. Add 240 µl Buffer MSL and 10µl Mag-Bind Particle E and mix the sample throughly by shaking or brief vortexing.

8. Add 330 µl absolute ethanol (room temperature, 96-100%) and mix thoroughly by vortexing at maxi speed for 20 seconds.

9. Transfer half volume of sample (415 µl) into a round bottom well process plate (500µl). And place the plate onto a magnetic separation device. Wait until all the magnetic beads are cleared from solution.

10. Remove and discard the supernatant. Transfer remaining sample (410µl) from step 9 into the plate. Repeat step 10-11 to collect magnetic beads.

11. Proceed the DNA binding, washing and elution by following step 10-21 in the standard protocol on page 6-7.

Trouble Shooting

Problem	Likely Cause	Suggestions
Low DNA yields	Incomplete resuspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	Inefficient cell lysis due to inefficient cell lysis	• Increase the lysiozyme 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 opetation	careful not remove the magnetic beads 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 stating 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.