HELINI
MagPure
Bacterial DNA
Purification Kit

Cat. No: 2504 – 96 Prep

Compatible with: Manual magnetic stand separator & Reputed open type Automatic DNA/RNA purification system

Introduction

The HELINI MagPure Bacterial DNA purification Kit is designed for rapid manual and automated purification of Bacterial DNA from human biological samples and culture. The Nucleic acid purified using the HELINI MagPure Nucleic acid purification kit contains high quality RNA and free of proteins, nucleases, and other contaminants or inhibitors. They are, therefore, suitable for direct use in many different downstream applications, such as qPCR (quantitative PCR), RT-qPCR (reverse transcription qPCR), and several other enzymatic reactions.

Intended Use

For the purification of Bacterial DNA from clinical samples and microbial cultures.

Principle and Procedure

The HELINI MagPure Bacterial DNA purification Kit uses magnetic-particle technology for Nucleic acid purification. The HELINI Biomolecules MagPure technology combines the speed and efficiency of nucleic acids purification with easy handling of magnetic particles. The purification process requires no phenol/chloroform extraction and needs very little hands-on time. The HELINI MagPure Magnetic Beads are highly reactive, super paramagnetic beads. The first step of the protocol lyses the sample, after which the nucleic acids can bind to the surface of the Magnetic Beads. The following three effective wash steps dispose of proteins, cell debris, and any residual contaminants, while the nucleic acids bound to the MagPure Magnetic Beads are transferred through the wash steps. High-quality nucleic acids are eluted into the nuclease-free water, and are ready for subsequent downstream processes.

Kit components

Components per plate	Qty	Storage
Magnetic beads	1.5ml	4ºC
Proteinase K	2ml	-20C
Lysis buffer	20ml	RT
Wash Buffer	120ml	RT
Elution Buffer	10ml	RT
Instruction manual		

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Discard sample and assay waste according to your local safety regulations.

Technical Assistance

For technical assistance and more information, please contact;

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Material required:

For Manual:

1. Micro pipettes – variable all standard ranges and micro tips
2. Magnetic stand separator
3. Micro centrifuge – 1.5/2ml rotors
4. 1.5/2.0 micro centrifuge tubes

For automation:

5. Reputed any make - Open system – Automated DNA/RNA purification system
6. Plastic wares & combes or tips compatible with automated purification system
7. Reagent reservoir - 5 Nos. Label them and use dedicated for that particular reagent only.
8. 8 channel variable micro pipette – range - 30 to 300µl
9. 8 channel variable micro pipette – range - 5 to 50µl
10. 8 channel variable micro pipette – range - 100 to 1000µl [optional]
11. Tips for micro pipettes – all above range.
12. 1.5/2.0 micro centrifuge tubes
13. Plate sealing film [optional]

Procedure - Manual method

1. Transfer 220µl of Lysis buffer into sterile 1.5ml centrifuge tube.

2. If you are using internal control template to monitor extraction efficiency, please pipette mix manufacturer indicated volume of internal control template.

3. Add 10µl of Magnetic beads. [Invert mix or vortex mix well the beads tube before pipetting]

4. Add 200µl of bacterial pellet suspended samples [Refer page-11]. Mix well by pipetting or pulse vortex for 10 seconds.

5. Add 20µl of Proteinase K and mix well by pulse vortex for 10 seconds. Brief spin to bring down the liquid to the bottom of the tube.

6. Incubate at 56C for 10min. [Intermediate brief vortex for two to three times is recommended].

7. Place it on the Magnetic stand separator for 10seconds. Discard the supernatant. [Decant or Pipette it out]

8. Takeout the tube from Magnetic stand separator and add 600µl of Wash buffer and vortex well for 10secs. Centrifuge the tubes for 5secs and incubate at room temperature for 10seconds.

9. Place on the Magnetic stand separator for 10seconds. Discard the supernatant completely. [Decant or pipette it out]

10. Takeout from the Magnetic stand and add 600µl of Wash buffer and vortex well for 10secs. Centrifuge the tubes for 5secs and incubate at room temperature for 10seconds.

11. Place on the Magnetic stand separator for 10seconds. Discard the supernatant completely. [Make sure there is no residual liquid in the tube, if any, use a micropipette to remove them]

12. Add 100µl of Elution buffer and vortex well for 10secs. Centrifuge the tubes 5 seconds and incubate at 56°C for 3mins.

13. Place on the Magnetic stand separator for 1min and carefully transfer elute in to fresh 1.5ml centrifuge tube.

14. Use the DNA immediately for qPCR/PCR or store at -20C/-80C for later use.

Recommendation:

Use 10µl for qPCR /PCR assay

Procedure

For Automation

1. Please refer the machine manufacturer recommended plastic wears and tips/combs for filling the reagents.
2. Use the following reagents volume, steps timings, mixing speed and heating steps;

Buffers	Volume Per well	Mixing Time	Heating Temp	Mixing Speed	Magnetic Beads pickup Time	Magnetic Beads Pickup speed
Lysis buffer	220µl	10min	65ºC	Moderate	10secs	Moderate
Wash buffer	600µl	2min	---	Moderate	10secs	Moderate
Wash buffer	600µl	1min	----	Moderate	10secs	Moderate
Elution buffer	100µl	3min	60ºC	Moderate	10secs	Moderate

Manufactured and Marketed by

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Sputum sample:

1. Add equal volume of 4M NaoH-NALC solution [freshly prepared] and mix well. Incubate for 45min to 1hour. Intermediate mixing is required for effective digestion.

2. Add equal volume of sterile distilled water and Mix well using sterile glass rod. Transfer into 15ml or 50ml centrifuge tube.

3. Centrifuge at 8000rpm for 10min. Discard the supernatant. Vortex well to dislodge the pellet.

4. Dissolve the pellet in 1 or 2ml of TE Buffer or PBS or sterile distilled water. [Volume can be adjusted based on the cell pellet]. Transfer 0.5ml [depends on the cell pellet turbidity] into fresh 1.5ml centrifuge tube.

5. Centrifuge at 8000rpm for 5min. Discard the supernatant. Vortex well to dislodge the pellet.

6. Use 200µl for DNA purification.

Culture sample:

1. Grow microbes in a suitable **5ml** sterile broth overnight [Minimum 16 hours]

2. Transfer 1.5ml to overnight culture into 1.5ml centrifuge tube and centrifuge at 10000rpm for 3min. Discard the supernatant.

3. Add 1ml of overnight culture into the same tube and centrifuge at 10000rm for 3min. Discard the supernatant.

4. Vortex the pellet for 5min to dislodge.

5. Add 300µl of Sterile water and use 200µl for DNA purification.