MagPure Stool DNA Kit

Introduction

MagPure Stool DNA Kit is specially designed for high throughput DNA extraction from stool samples. It can get high purity microbial DNA from stool samples (≤200mg). This kit is based on magnetic beads purification and unique inhibiting factors adsorption technology, no phenol-chloroform extraction or alcohol precipitation. It can adsorb humic acid and other inhibiting factors in the solution efficiently. DNA can be directly used for downstream applications such as PCR, Viral DNA testing, bacterial DNA testing, ect. MagPure LQ Kits buffers and purify process are specially designed for automatic liquid handling workstations and also for manual extraction.

Kit Contents

Product	D636401	D636402	D636404
Preps per Kit	48 Preps	96 Preps	400 Preps
MagPure Particles N	1.7 ml	3.4 ml	14 ml
2ml Bead Tubes	48	96	400
RNase A	10 mg	20 mg	75 mg
Proteinase K	24 mg	48 mg	180 mg
Protease Dissolve Buffer	3 ml	5 ml	20 ml
Buffer ATL	60 ml	110 ml	300 ml
PVP-10	1.2 g	2.2 g	6 g
Buffer PCI	50 ml	100 ml	300 ml
Buffer MLE*	30 ml	60 ml	180 ml
Buffer GW1*	22 ml	44 ml	132 ml
Elution Buffer	20 ml	20 ml	60 ml

Storage and stability

MagPure Stool DNA Kits components are guaranteed for at least one year when stored at room temperature. If any precipitates form in the Buffer ATL, warm at 37℃ to dissolve. Proteinase K dry powder is preserved at room temperature. After dissolving, Proteinase K needs to be stored at -20℃. MagPure Particles N should be stored at 2~8℃.

Materials and Equipment to be Supplied by User

- Heat block or water bath capable of 70℃
- 75% and 100% ethanol
- Add Protease Dissolve Buffer to the Proteinase K (20mg/ml) and store at -20~8℃.
- Add Protease Dissolve Buffer to the RNase A (15mg/ml) and store at -20~8℃.
- Add 28ml (48 Preps) or 56ml (96 Preps) or 168ml (400 Preps) 100% ethanol to Buffer GW1 and store at room temperature
- Add 20ml (48 Preps) or 40ml (96 Preps) or 120ml (400 Preps) Isopropanol to Buffer MLE and store at room temperature
- Magnetic Particles N should be shake violently for 1~2 minutes to be homogeneous.

Protocol

Sample Preparation (lysis by beads)

This protocol is used for extracting high purity DNA from 100~150mg stool samples.

1. Transfer 100~150mg stool sample to 2ml Bead tube. For liquid samples, pipette ~0.15ml samples. Cut the end of pipet tip to make pipetting easier.
2. Add 0.6ml Buffer ATL/PV-10 and 0.6ml Buffer PCI into the sapmle. Place on a bead beater machine or vortex at maximum speed for 10 min. We recommend to use FastPrep-24, Tissue Lyser, ect. Processing time depends on sample input and bead beater.

   Note: Add PVP-10 powder into Buffer ATL before use, dissolve completely by up side down.

3. Incubate sample at 65℃ for 20 minutes. This step makes bacteria lyse more completely.
4. Centrifuge at 13,000 x g for 5 minutes and follow below purification process.

Purification Process

Protocol A: Manual Single Tube Process

1. Transfer 400µl supernatant to a new 2ml centrifuge tube.
2. **Add 10 µl RNase A** and mix thoroughly, Sit at room temperature for 5~10 minutes.
3. **Add 30 µl MagPure Particles N, 20 µl Proteinase K and 600 µl Buffer MLE** to the samples. Mix thoroughly by vortex for 10 minutes.
4. Place the tube to the magnetic stand for 2~3 minutes until the beads have formed a tight pellet. Then remove the supernatant.
5. **Add 600µl Buffer GW1** and vortex for 15 seconds to re-suspend beads. Place the tube to the magnetic stand for 1 minute until the beads have form a tight pellet. Then remove the supernatant.
6. **Add 600µl 75% ethanol,** and vortex for 15 seconds to re-suspend beads. Place the tube to the magnetic stand for 1 minute until the beads have form a tight pellet. Then remove the supernatant.
7. Repeat step 6.
8. Centrifuge shortly to collect liquid on the tube. Place the tube to the magnetic stand and remove all the liquid carefully.
9. Dry on air for 10 minutes.
10. **Add 50~100µl Elution Buffer to the sample,** re-suspend the beads by vortex. Incubate at 55°C for 10 minutes by shaking. If there is no shaking device, vortex 2~3 times to mix DNA with magnetic particles.
11. Place the tube to the magnetic rack for 2 minutes. Transfer the supernatant containing the purified DNA to a clean 1.5ml centrifuge tube.

Protocol B: High Throughput Process

1. Transfer 300µl supernatant to a new 2.2ml 96 well deep well plate.
2. Add 10µl RNase A and mix thoroughly and sit at room temperature for 10 minutes.
3. **Add 25µl MagPure Particles N, 20µl Proteinase K and 450µl Buffer MLE(Isopropanol added)** to each well. Shake at 800~1200rpm for 10 minutes to mix thoroughly. (optimized the shaking speed with same volume water before use. With high speed to mix but liquid do not overflow.)
4. Place the plate to 96 well magnetic stand for ~5 minutes, then remove the supernatant.
5. **Add 600µl Buffer GW1 to each well.** Re-suspend the beads by shaking at 900~1,200 rpm for 2 minutes. Place the plate to the magnetic rack for 2 minutes, then remove the supernatant.
6. **Add 600 µl 75% ethanol to each well.** Re-suspend the beads by shaking at 900~1,200 rpm for 2 minutes.Place the plate to the magnetic rack for 2 minutes, then remove the supernatant.
7. **Add 600 µl 75% ethanol to each well.** Re-suspend the beads by shaking at 900~1,200 rpm for 2 minutes. Place the Plate to the magnetic rack for 2 minutes, then remove the supernatant.
8. Place the Plate to the magnetic rack, add 250~300µl ddH2O to the well, do not disperse the magnetic pellet, sit for 5~10 seconds, then remove the water. [for 4-8 channol machine, process one set of the samples, then another set of the samples]
9. **Add 100 µl Elution Buffer to the samples.** Re-suspend the beads by shaking at 1,200rpm for ~10 minutes at 50~55°C.
10. Place the plate to 96 well magnetic stand for 3 minutes, then transfer the supernatant containing the purified DNA to a new 96 well plate.