MagPure Circulating DNA Rich Maxi Kit

Introduction

The MagPure Circulating DNA Rich Maxi Kit is designed to rich and extract 100bp~500bp circulating DNA from 5 ml cell-free body fluids (such as plasma, serum), and remove fragments above 500bp. Machine reaction time is only 90 minutes. Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications such as PCR and next generation sequencing.

Introduction

This product is based on the purification method of high binding magnetic particles. The sample is lysed and digested under the action of lysate and Protease. DNA is released into the lysate. After adding magnetic particles and binding solution, DNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing buffer to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer.

Kit Contents

Product	1292750	12927200
Preps per Kit	50 Tests	200 Tests
MagPure Particles G	20 ml	80 ml
MagBind Particles	14 ml	58 ml
Selection Solution	100 ml	400 ml
Proteinase K	300 mg	1.2 g
Protease Dissolve Buffer	25 ml	100 ml
Buffer SDS(20%)	15 ml	60 ml
Buffer MLK	300 ml	3 x 450 ml
Buffer BST1	225 ml	2 x 450 ml
Buffer MKW1	225 ml	2 x 450 ml
Buffer MW2*	50 ml	2 x 100 ml
Buffer AE	10 ml	30 ml

Storage Conditions

MagPure Particles G, MagBind Particles and Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Reagents to Be Supplied by User

1. **Prepare the Proteinase K working solution:** Add 15ml (50 Test) or 60ml (200 Test) /60ml Protease Dissolve Buffer to the vial of the lyophilized Proteinase K to be a concentration of 20mg/ml. The Proteinase K working solution should be stored at -20°C. Repeated freezing and thawing should be avoided.
2. **Buffer MW2 is supplied as a concentrate.** Before using for the first time, add 200ml (50 Tests) or 2 x 400ml (200 Tests)100% ethanol as indicated on the bottle to obtain a working solution.
3. Shaking MagPure Particles G and MagBind Particles thoroughly before use.

Protocol A: for 5ml serum/plasma sample

1. Add 5ml plasma/serum to a sterile 15~50ml centrifuge tube. **Add 250µl Proteinase K and 250µl Buffer SDS(20%) to the sample,** and vortex for 5 seconds. Incubate at 55°C for 30 minutes.
2. **Add 6ml Buffer MLK, 250µl MagBind Particles and Select Solutions (1000µl, 1250µl or 1500µl)** to the sample, vortex for 10 minutes. Centrifuge at 3,000~5,000 x g for 10 minutes. Transfer the supernatant to a new 15ml centrifuge tube. (MagBind Particles adsorb large fragments in this step, discard the MagBind Particles in this step.)
   Note: 1000µl Select Solutions remove fragments above 1kb, 1250µl Select Solution remove fragments above 600bp and 1500µl Select Solution remove fragments above 500bp. This result is get according to adding extra DNA Marker fragment test. Users can adjust the volume according to test results.
3. **Add 3.8ml Buffe BST1 and 360µl MagPure Particles G to sample,** Inverting Mix for 10 ~15 minutes. Centrifuge at 3,000~5,000 x g for 5 minutes. Discard the supernatant. (MagPure Particles G adsorb 100~500bp cell free DNA fragments in this step.)
4. **Add 1.5ml Buffer MKW1**, vortex for 20 seconds to mix the particles thoroughly. Transfer to a new 2.0ml centrifuge tube. Place the tube to the magnetic rack for 2 minutes, until the MagPure Particles G have formed a tight pellet, then remove the supernatant.
5. Rpeat step 4 once.
6. **Add 1.5ml Buffer MW2 (make sure ethanol was added), and vortex for 20 seconds.** Place the tube to the magnetic rack for 2 minutes, then remove the supernatant.
7. Repeat step 6 once.
8. **Add 1.5ml Absolute ethanol and vortex for 20 seconds.** Place the tube to the magnetic rack for 2 minutes, then remove the supernatant.
9. Spin shortly to collect liquid on the tube, place the tube to the magnetic rack, remove all the liquid carefully. Dry at 55°C for 15 minutes.

10. **Add 70µl Buffer AE, Low TE or ddH_2O (preheated to 55°C) to the tube,** and shaking for 6 minutes to mix the particles thoroughly.

11. Place the tube to the magnetic rack for 2 minutes, until the Particles have formed a tight pellet. Transfer the supernatant containing the purified DNA to a 1.5ml centrifuge tube.

12. **Add another 30ul Buffer AE, Low TE or ddH_2O (preheated to 55°C) to the tube,** and shaking for 2~3 minutes to mix the particles thoroughly.

13. Place the tube to the magnetic rack for 3 minutes, until the Particles have formed a tight pellet. Transfer the supernatant containing the purified DNA to a 1.5ml centrifuge tube (Step 11).