MagPure Plasmid Mini Kit

Introduction

The MagPure Plasmid purification system uses the paramagnetic bead technology for high-throughput preparation of high-copy or low-copy plasmid DNA from E. coli cells. Theis kit also be used with fosmid and BAC vector-based constructs. The system uses alkaline lysis followed by a MagPure purification to differentially bind plasmid DNA to paramagnetic beads. While the DNA is bound to the beads, contaminants can be rinsed away using a simple washing procedure. Because MagPure uses magnetic separation technology, the protocol does not require vacuum filtration. This makes kit extremely amenable to automation. Plasmid DNA purified with this system is most commonly used in Sanger Sequencing and PCR amplification.

Kit Contents

Product Number	P181102	P181103	P181104
Purification Times	100 Preps	500 Preps	5000 Preps
RNase A	10 mg	50 mg	2 x 250 mg
Buffer P1	30 ml	150 ml	2 x 750 ml
Buffer P2	30 ml	150 ml	2 x 750 ml
Buffer N3	30 ml	150 ml	2 x 750 ml
Buffer PW1	35 ml	180 ml	2 x 900 ml
MagPure Particle NB*	2.2 ml	11 ml	2 x 60 ml

Storage and Stability

RNase A and MagPure Particle NB should be stored at 2–8°C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15–25°C) does not affect its 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. After addition of RNase A, Buffer P1 is stable for 6 months when stored at 2–8°C.

Materials and Equipment to be Supplied by User

- Add 28ml (100Preps) or 130ml (500Preps) or 2 x 700 ml (5000 Preps) isopropanol to MagPure Particle NB and store at room temperature.
- Add RNase A to the bottle of Buffer P1 and store at 2-8°C

Protocol

1. Pipette 1.3~1.7mL 2xYT bacterial growth media containing the appropriate antibiotic (see Recommendations below) into each well of a 2.2 mL deep-well culture block.Inoculate each well with a single plasmid containing E. coli bacterial colony. Cover the plate with a gas permeable seal and shake at 300 RPM and 37°C.

2. **Pellet bacterial cultures by centrifuging culture plates at 2,500 × g for 10 minutes.** After centrifugation, remove the seal and invert the block to decant the media away from the cell pellets. Blot the inverted block on a paper towel to remove excess media.

3. **Add 250µl Buffer P1 and thoroughly resuspend cell pellets by vortexing, shaking or pipette mixing.**
   Ensure that RNase A has been added to Buffer P1. Pipette mixing and shaking are most often used for automated processes. Pipette mix at least 20 times (if possible, pipette mix 5 times at 4 different locations in the well) for an even resuspension. Alternatively, shake 4 minutes at 600 to 1,200 RPM on a shaker. Shaking speeds will vary, depending on the orbit of the shaker. Vortexing will take 2 to 3 minutes on a high setting. The cell pellet should be completely resuspended so that the mixture appears homogeneous and has no cell clumps.

4. **Add 250µl Buffer P2 and allow the samples to lyse for 5 minutes.**
   Shake 5 minutes at 300-600 RPM. Alternatively, gently pipette mix two times, and then allow the samples to sit for 5 minutes for a complete lysis. Vigorous pipette mixing is not recommended, as BACs can be easily sheared. Do not allow samples to lyse for longer than 10 minutes. If a white precipitate is seen in the P2 solution prior to addition, warm the bottle in a 37°C water bath or under hot running water, shaking periodically, until the precipitate dissolves. It is recommended that gloves are worn when handling P2 , as it is a basic solution.

5. Add 250µl Buffer N3 and shake 10 minutes on an orbital or linear shaker to neutralize samples.

   Addition of Buffer N3 neutralizes the solution and precipitates proteins and cellular debris, creating a white flocculent. BACs must be shaken to help release the large template from the flocculent; the same orbital shaker can be used for overnight culture (room temperature or 37°C are both fine). It is recommended that gloves are worn when handling Buffer N3 .

6. Following neutralization, centrifuge samples at 4,700 × g for 20 minutes to pellet the flocculent.

   Pelleting of the flocculent allows for transfer of a greater percentage of the lysate and cleaner plasmid. A 20 minute spin is usually sufficient to pellet the flocculent, but if the pellet is not tightly bound to the bottom of the well, the centrifugation time should be increased.

7. Transfer 500µl of the clear lysate to a 2.0 mL deep well plate. This transfer is the most critical step of the process.

   The transfer volume should be chosen so that the supernatant is free of cellular contaminants (flocculent) for optimal results. For best results, use slow aspiration speeds and remove the clear lysate from the top of the well. The aspiration height used for this transfer step will most likely need to be optimized to avoid disturbing the flocculent pellet.

8. Add 250µl of Bind Beads N (Isopropanol added) to the sample, and Pipette mix 20 times or shaking for 5 minutes.

   Bind Beads N contains magnetic particles and Isopropanol. As soon as both the magnetic particles are added, the beads may begin to fall out of solution if the sample is not pipette mixed immediately. The liquid should appear homogeneous after mixing. Shaking samples for 5 min, instead of pipette mixing, may result in higher yield.

9. Place the deep well plate on an Magnet Plate and allow beads to separate for 2 minutes.

   With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

10. Recommended: Wash the beads by adding 0.3ml Buffer PW1, resuspend the beads by pipette mix 10 times or shaking for 1min.

This step is necessary to remove trace RNA and trace nuclease activity when using endA+ strains, such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content.

11. Place the deep well plate on an Magnet Plate and allow beads to separate for 1 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

12. Wash the beads by adding 0.5 ml 75% ethanol, resuspend the beads by pipette mix 10 times or shaking for 1min.

13. Place the deep well plate on an Magnet Plate and allow beads to separate for 1 minutes. With the plate on the Magnet Plate, perform the aspiration, and then discard the supernatant from the plate.

14. Repeat step 12~13 once.

15. Dry the BAC, Fosmid, or plasmid samples at 50°C for 8 minutes.
    Plates can also be dried at room temperature for 10~15 minutes. For best results, do not over dry BACs.

16. Add 30~100µl Water or Buffer TE to each well of the plate, and then incubate for 5 minutes at 37°C.

    Vortex or shake the plate for 60~120 seconds after incubating for 5 minutes at 37°C to fully elute the plasmid from the beads. For large templates, especially BACs or Fosmids, it is helpful to let the plates sit for 5 to 10 minutes after vortexing to allow the large templates extra time to dissociate from the beads.

17. Place the deep well plate on an Magnet Plate and allow beads to separate for 2 minutes. With the plate on the Magnet Plate, perform the aspiration, and then transfer the supernatant containing DNA into a new plate.