MagPure Plasmid EF 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	P181402	P181403	P181404
Purification Times	100 Preps	500 Preps	5000 Preps
RNase A	10 mg	30 mg	2 x 160 mg
Buffer P1	30 ml	150 ml	2 x 800 ml
Buffer P2	30 ml	150 ml	2 x 800 ml
Buffer LEN3	20 ml	80 ml	800 ml
Buffer LN4	90 ml	400 ml	4 x 980 ml
MagPure Particles	3.5 ml	17 ml	3 x 60 ml

Storage and Stability

RNase A and MagPure Particles 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 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.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. If any precipitates form in the buffers,warm at 37℃ to dissolve. 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 the vial of 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 260µl 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 260µl 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 130µl Buffer LEN3 and shake 10 minutes on an orbital or linear shaker to neutralize samples.**
   Addition of Buffer LEN3 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 LEN3 .

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. If the cleare lysate is less than 500µl, it is recommended to increase the volumes of Buffers P1, P2, and LEN3 used in subsequent preparations.

8. **Add 250µl of Buffer LN4、150µl Isopropanol and 30µl MagPure Particles to the sample, and Pipette mix 10-20 times.**
   MagPure Particles, Isopropanol and Buffer LN4 can be premixed before use.

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. **Wash the beads by adding 0.5ml Buffer LN4, resuspend the beads by pipette mix 10 times or shaking for 1min.**

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 0.7ml 80% 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. **Wash the beads by adding 0.7ml 80% ethanol, resuspend the beads by pipette mix 10 times or shaking for 1min.**

15. 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.
    It is recommended to let sit for 1 minute then remove any remaining liquid from the wells.

16. 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.

17. Add 50~75µl 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 ~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.

18. 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 into a new plate.