Puramag™ Plasmid DNA Isolation Kit

User Manual

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Introduction

The Puramag™ Nucleic Acid Isolation System is a simple, high-throughput magnetic bead based DNA and RNA isolation system. By using magnetic microparticles coated with carboxyl groups, Puramag™ allows for rapid and reliable isolation of high quality nuclei acid from a multitude of sources. The use of magnetic beads obviates the need for columns and centrifugation thereby allowing for easy automation on most robotic platforms.

Product Description

The Puramag™ Plasmid DNA Isolation Kit provides rapid isolation of high quality plasmid DNA, BACs, PACs, and cosmids after alkaline-SDS lysis of bacterial cells. The carboxyl coated magnetic beads provide efficient binding of plasmid DNA which is then easily eluted with deionized water or a low salt buffer (Tris-HCl, TE).[1] The system is easily automatable after bacterial lysis and reproducibly yield >10µg high quality plasmid DNA from 1ml E. Coli cultures.* The resulting plasmid DNA after Puramag™ isolation is ready for subsequent downstream applications including digestion and fluorescent DNA sequencing.

*Yield depends on a number of factors including but not limited to the specific plasmid, bacterial growth conditions (medium, rotation rate, temperature, and time), bacterial strain, and plasmid copy number. For best results, we recommend that 1ml E.Coli cultures have an O.D.¬600 of 1.0-6.0.

References

1. Hawkins, T.L., et al. (1994). DNA purification and isolation using a solid-phase. Nucl. Acid Res. 22(21):4543-4

Kit components

Product	Puramag™ plasmid DNA Isolation Kit		
Size	100 rxns	1,000 rxns	10,000 rxns
Cat. #	PMB-100	PMB-101	PMB-102
Solution 1	5 ml	50 ml	500 ml
Solution 2	5 ml	50 ml	500 ml
Solution 3	5 ml	50 ml	500 ml
Puramag™ Bead Solution	1ml	10 ml	100ml
Elution Buffer	5 ml	50 ml	500 ml
Binding Buffer	10 ml	100 ml	1 L
Wash I	20 ml	200 ml	2 L
Wash II	40 ml	400 ml	4 L

Storage Conditions: Keep Solution 1 (after RNase A addition) at +4°C. The remaining items can be stored at room temperature.

Protocol

Materials to be supplied by the user
- Table Top Centrifuge
- Magnetic Separation Device
- RNase A (100mg/ml)
- 1.5 ml - 2 ml centrifuge tubes

Setup
- Before using Solution I, add RNase A into Solution I to a concentration of 100µg/ml, Keep at +4°C.
- Make sure Solution 2 solution does not have any precipitate. If precipitate is present, heat at 37°C for 5 minutes or until precipitate disappears.

Manual Procedure

1. Pellet 1 mL overnight bacterial cultures by centrifugation for 5 minutes at 3000 x g on a table top centrifuge.

2. Decant supernatant and resuspend pellet in 50µl Solution 1. Fully resuspend the cells by vortexing or pipetting.

3. Add 50µl Solution 2, mix gently, and incubate at room temperature for 30 seconds or until solution clears. Max incubation time should not exceed 5 minutes.
   Do not vortex or mix vigorously after Solution II addition as it can shear chromosomal DNA.

4. Add 50µl Solution 3, mix gently for 10 seconds, and incubate at room temperature or on ice for 1 minute. Max incubation time should not exceed 10 minutes, White precipitate should form after addition of Solution 3.
   Do not vortex or mix vigorously after Solution 3 addition as it can shear chromosomal DNA.

5. Centrifuge at 3000 x g for 10 minutes to pellet debris.

6. Pipet 100µl of the supernatant (plasmid DNA) into new sample tubes.

7. Add 10µl of Puramag™ Bead solution.

8. Add 100µl binding buffer and mix by pipetting incubate at room temperature for 2 minutes.

9. Place sample on the magnetic separation device and wait for the supernatant to clear.

10. While sample tubes are situated on the magnetic separation device, aspirate supernatant and discard.

11. Add 200µl wash buffer I and incubate for 30 seconds.
    Do this step while the sample tubes are situated on the magnetic separation device. There is no need to resuspend the beads.

12. Aspirate and discard.
    It is important to do this step while the sample tubes are situated on the magnetic separation device.

13. Add 200µl wash buffer II and incubate for 30 seconds.

14. Aspirate and discard.

15. Repeat steps 13 and 14.

16. (Recommended) Quick spin tubes and place back on magnetic separation device and wait for supernatent to clear. Aspirate the residual wash buffer.

17. With the sample tubes off the magnetic separation device, resuspend magnetic beads in 50µl of ELUTION BUFFER or deionized water / TE / Tris Acetate / Tris-Hcl. Incubate at room temperature for 5 minutes.
    Make sure the beads are fully resuspended after mixing. Vanying elution volumes can be used if higher or lower concentrations are desired.

18. Place sample tubes onto the magnetic separation device and wait 5 minutes or until the solution clears. While keeping the sample tubes on the magnetic separation device, transfer 45µl of cleared elution onto a new plate.

    5 µl is left behind to prevent transfer of beads as it can interfere with subsequent reactions. If beads do transfer, place samples back onto original plate and re-transfer onto a new plate.

Troubleshooting Guide

Please consult this troubleshooting guide if any problems arise. If problems cannot resolved through use of this guide, please contact technical support at MCLAB, toll free, at (888) MCLAB-88. You can also chat live with technical support at www.mclab.com

Low DNA yield

Problems	Solutions
Bacterial cultures not fully grown, overgrown, or not fresh	Bacterial cultures not fully grown, overgrown, or not fresh
Incomplete cell lysis	-Cell density could be too high. Use less cultures or more Solution 2. -Inadequate mixing could also result in incomplete cell lysis. Make sure sample is thoroughly mixed and that solution turns clears after addition and mixing of Solutio 2.
Low copy number plasmids	DNA yield depends on a number of including plasmid copy number. If low copy number plasmids used, repetitions may need to be carried out in order to yield satisfactory amounts of low copy number plasmid DNA.

No DNA yield

Problem	Solution
Incorrect buffer s or buffer concentrations used	Make sure binding buffer is used in the precipitation and binding of plasmid DNA onto the Puramag™ beads. Make wash buffer I and II are used during the DNA washes.

Poor DNA quality

Problems	Solutions
Cell lysis time was too long	Make sure incubation after Solution 2 addition does not last longer than 5 minutes.
Nicked or she ared plasmid DNA	Do not vortex sample after Solution 2 and Solution 3 addition. Also, make sure isolated plasmid DNA is properly buffered such as in TE, pH 8.0.
Genomic/High molecular weight DNA contamination	Do not vortex the cell lysates after addition of Solution 2 and Solution 3. Vortexing or aggressive mixing of lysates will shear genomic DNA resulting in contamination of plasmid DNA.

RNA contamination

Problem	Solution
Insufficient RNase A in Solution 1	Make sure RNase A is added into Solution 1 prior to use at a final concentration of 100µg/ml. Solution 1 after RNase A addition should be kept on ice during use and stored at +4°C. If Solution 1 after RNase A addition has not been properly stored, discard and use a new aliquot with fresh RNase A.

Plasmid DNA not loading onto agarose gel

Problems	Solutions
Not enough DNA loading dye	Make sure enough DNA loading dye is added prior to loading DNA onto gels.

Poor downstream performance of plasmid DNA.

Problems	Solutions
Protein contamination	Make sure bacterial lysate is clear after Solution 3 addition and subsequent 10 minute spin.
Ethanol contamination	Make sure all the ethanol is dried prior to plasmid DNA elution.
Salt contamination	Make sure DNA is thoroughly washed with 70% ethanol. Extra washes can be carried out to ensure clean DNA.
Magnetic bead contamination	Make sure no beads are transferred along with isolated plasmid DNA onto the final plate.