MagPure Plasmid EF Mini Kit

Introduction

This product employs magnetic-bead purification technology and is suitable for isolating high-concentration, high-yield, low-endotoxin, transfection-grade plasmid DNA from 1–5 mL bacterial cultures. The purified plasmid yield is up to 15 μg, with an endotoxin level of <1 EU/μg. Plasmid DNA purified by this system is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, cell transfection , cloning, PCR and other applications.

Kit Contents

	P181400	P181402	P181403
Purification Times	20 preps	200 preps	500 preps
RNase A	5 mg	10 mg	20 mg
Buffer P1	15 ml	60 ml	150 ml
Buffer P2	15 ml	60 ml	150 ml
Buffer LEN3	10 ml	30 ml	80 ml
Buffer MN4	20 ml	70 ml	180 ml
Buffer PW1	40 ml	140 ml	350 ml
Buffer EWB	40 ml	140 ml	350 ml
Elution Buffer	10 ml	30 ml	60 ml
MagPure Particles	2 ml	7 ml	17 ml

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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 24 months under these conditions. 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 0.5 ml Buffer P1 to the vial of RNase A and pipette to dissolve. Transfer the entire solution to the Buffer P1 bottle. Store the prepared Buffer P1/RNase A mixture at 2–8°C for up to 6 months.

Protocol 1. Single-Tube Procedure

1. **Centrifuge 1–5 ml bacterial culture at 13,000 × g for 1 minute to pellet the cells. Discard the medium and tap the inverted tube on absorbent paper to remove all residual liquid.**
   Culture method: Under aseptic conditions, pick a single colony with a sterile toothpick and inoculate it into 1–5 ml LB medium containing the appropriate antibiotic. Incubate at 37°C with shaking at 250–300 rpm for 12–14 hours. The culture tube or flask should preferably have a capacity 4–5 times the culture volume. Because 2× YT or TB medium produces very high cell densities, do not use more than 2.5 mL culture. Cell growth in 2× YT or TB is too rapid for optimal plasmid replication; avoid these media whenever possible. For low-copy-number vectors, it can proceed up to 15 ml culture; increase the volumes of Buffer P1, Buffer P2, and Buffer LEN3 by 1.5-fold.

2. **Add 260 µl Buffer P1/RNase A mixture. Resuspend the bacterial pellet thoroughly by vigorous vortexing or pipetting.**
   Ensure that RNase A has been added to Buffer P1 before use. Complete resuspension is critical for optimal yield; no cell clumps should remain. If vortexing does not completely disperse the pellet, pipette up and down several times.

3. **Add 260 µl Buffer P2 to the resuspended cells. Mix by inverting 8–10 times, or until the cells are completely lysed.**
   Do not vortex or shake, as this may cause genomic DNA contamination. After mixing, the lysate should become viscous and clear. For a large bacterial pellet, incubate at room temperature for 2–3 minutes and invert several additional times to ensure complete lysis. When processing 5 ml culture, the lysate will be extremely viscous because of the high cell density. Additional gentle inversions and rotations may be required to obtain a homogeneous, clump-free lysate. Do not exceed a total lysis time of 5 minutes.

4. **Add 130 µl Buffer LEN3. Immediately invert 10–15 times until a flocculent suspension forms, then centrifuge at 13,000 × g for 10 minutes.**

5. **Transfer 500 µl supernatant to a new 1.5 ml microcentrifuge tube.**

6. **Add 300 µl Buffer MN4 and 30 µl MagPure Particles. Mix by inverting 15–20 times and**

incubate for 5 minutes, inverting several times during incubation. Place the tube on a magnetic rack for 1 minute, then aspirate or decant all liquid.

For an expected total yield of no more than 10 µg, add 20 µl MagPure Particles. For an expected yield of 10–20 µg, add 30 µl MagPure Particles.

7. Add 500 µl Buffer PW1 and vortex for 15–30 seconds. Place on the magnetic rack for 1 minute and discard the liquid.

8. Add 500 µl Buffer EWB and vortex for 15–30 seconds. Place on the magnetic rack for 1 minute and discard the liquid.

9. Add 750 µl 80% ethanol and vortex for 15 seconds. Place on the magnetic rack for 1 minute and discard the liquid.

10. Add 750 µL 80% ethanol and vortex for 15 seconds. Place on the magnetic rack for 1 minute and discard the liquid.

11. Briefly centrifuge, remove all residual liquid, and air-dry for 10 minutes.

12. Add 50–100 µL Elution Buffer and mix for 10 seconds. Incubate at room temperature for 5 minutes, vortexing several times during incubation. Place on the magnetic rack for 2 minutes, then transfer the DNA-containing eluate to a new 1.5 mL microcentrifuge tube.

Protocol 2. Operation on a 96-Channel Nucleic Acid Extractor

1. Culture in a 96-well plate: Add 1.3 ml LB or YT medium to each well of a 2.2 ml deep-well plate (not supplied). Inoculate each well with a single colony, seal with a gas-permeable film, and incubate at 37°C with shaking at 220–280 rpm for 16 hours to amplify the plasmid.

2. Centrifuge at 2,500 × g for 10 minutes to pellet the bacteria. Discard the medium, invert the plate, and gently tap it on absorbent paper to remove all residual liquid. At this point, the samples may be sealed and frozen at −20 to −80°C for later processing.

3. Add 260 µl Buffer P1/RNase A mixture to each well. Resuspend the bacterial pellet by vortexing at maximum speed or by pipetting.

   Ensure that RNase A has been added to Buffer P1 before use. Complete resuspension is essential for high yield; no cell clumps should remain. When using a liquid-handling workstation or plate vortex mixer (e.g., IKA MS3), vortex at maximum speed for 3–5 minutes or until the pellet is fully resuspended. For cultures grown in 24-well plates, combine the resuspended cells from four 24-well plates into one 96-well plate.

   For fully automated extraction on a liquid-handling workstation, magnetic beads may be used

to remove impurities. Add 30 µL MagPure Particles to the resuspension. Buffer P1/RNase A and MagPure Particles may be premixed at a ratio of 280 µL Buffer P1 to 30 µL MagPure Particles; the mixture may be stored at 2–8°C for 6 months. When magnetic-bead impurity removal is used, no more than 3 mL LB culture is recommended.

Liquid-handling workstation or 96-well plate vortex mixer (IKA MS3): Shake at maximum speed for 5–10 minutes to fully resuspend the bacteria.

4. **Add 260 µl Buffer P2 to each well. Apply a sealing film, gently invert the plate 10–15 times, briefly centrifuge to collect droplets from the well openings, and incubate at room temperature for 2–3 minutes.**

   Liquid-handling workstation or 96-well plate vortex mixer (IKA MS3): Shake at 400–700 rpm for 3–5 minutes.

5. **Remove the sealing film and add 130 µl Buffer LEN3 to each well. Reseal the plate, immediately invert 10–15 times, and shake at 1,000–1,200 rpm for 2 minutes to disperse large precipitate aggregates.**

   Liquid-handling workstation or 96-well plate vortex mixer (IKA MS3): Shake at 600–800 rpm for 3 minutes, then at 1,000–1,200 rpm for 2 minutes to break large aggregates into smaller flocs or proteinaceous precipitates for easier clarification in the next step.

6. **Select an appropriate method to remove the precipitated impurities formed after neutralization.**

   Centrifugation: Centrifuge at 4,000–4,500 × g for 20 minutes to pellet the impurities. Using a pipette or liquid-handling workstation, slowly aspirate the supernatant (~500 µL) from the highest liquid level. Twenty minutes is usually sufficient to pellet the flocculent material. If the flocs are not firmly attached to the bottom of the wells, increase the centrifugation time.

   Centrifugal filtration: Place a 96-well Filter Plate B (sold separately, M064-01) on a 1.6–2.2 mL 96-well plate. Transfer the entire mixture to the filter plate and centrifuge at 4,000 × g for 3 minutes to remove impurities and obtain a clear filtrate.

   Vacuum filtration: Place the 96-well filter plate (sold separately, M064-01) in a vacuum manifold, with a 1.6–2.2 mL 96-well collection plate underneath. Transfer the entire mixture to the filter plate and apply vacuum to obtain a clear filtrate.

   Magnetic-bead clarification: When using magnetic beads for impurity removal, 30 µL MagPure Particles must be added in Step 3. After neutralization, the magnetic particles bind to the flocculent precipitate to form a magnetic pellet. Place the sample on a 96-well magnetic rack for 3–5 minutes to capture the magnetic particles and impurities on the sidewall or bottom, then slowly transfer an appropriate volume of supernatant (~500 µL).

7. Prepare the prefilled reagent plate: Invert the 96-well plate to fully resuspend the magnetic particles. Return it upright for 1 minute, then remove the outer sealing bag and sealing film.

8. Transfer 500 µl supernatant to the sample plate. Insert the magnetic-rod sleeve into the instrument and load the 96-well plate, positioning well A1 at the inner-left corner.

9. After approximately 30 minutes, the extraction is complete. Remove the 96-well plate and magnetic-rod sleeve.

10. Transfer the DNA to 1.5 mL microcentrifuge tubes and store the purified product at −20 to 8°C.

Recommend program on Magmix 16/32

NO	Step	well	volume	Mix time		waiting		Magnetic time			Magnetic absorption	heating	
				time	speed	time	place	lift	Fluid level	bottom		plate	temp
1	Capture	4	500	0.5 min	8	0	0	60 s	0	0	Auto	/	/
2	Bind	1	800	7 min	8	0	0	60 s	0	0	Auto	/	/
3	Wash 1	2	600	1 min	8	0	0	50 s	0	0	Auto	/	/
4	Wash 2	3	600	1 min	8	0	0	50 s	0	0	Auto	/	/
5	Wash 3	4	900	1 min	8	0	0	50 s	0	0	Auto	/	/
6	Wash 4	5	900	1 min	8	0	0	50 s	0	0	Auto	/	/
7	Dry	6	600	0	0	8 min		0	0	0	Auto	/	/
8	Elute	6	100	6 min	8	0	0	60 s	0	30	Auto	/	/
9	Release	3	500	0.5 min	8	0	0	0	0	0	Auto	/	/