Contents

Important Notes:

The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 3 to 5 times. Please contact our customer service for further information and reference the table below for the commonly used plasmids,

Plasmid	Origin	High copy	Low copy
pACYC	P15A		10-12
pSC101	pSC101		5
pSuperCos	pMB1		10-20
pBR322	pMB1		15-20
pUC	Muted pMB1	500-700	
pGEM^{R}	Muted pMB1	300-400	
pBluescript^{R}	ColE1	300-500	

Introduction

Key to the kit is our proprietary Buffer RET system that allows the high efficient binding of DNA to our ezBind^{TM} matrix while endotoxin, proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or TE buffer. The purified DNA is ion exchange resin residues free which enable the high performance of downstream applications such as transfection, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations.

Storage and Stability

Buffer A1 should be stored at 4°C once RNase A is added. All other materials can be stored at room temperature. The Guaranteed shelf life is 18 months from the date of purchase.

Kit Contents

Catalog Number	PD2568-01	PD2568-02
Preps	10	25
Maxi Columns	10	25
Syringe Filters	10	25
Buffer A1	110 mL	220 mL
Buffer B1	110 mL	220 mL
Buffer N3	60 mL	120 mL
Buffer RET	110 mL	220 mL
Elution Buffer	25 mL	60 mL
DNA Wash Buffer**	50 mL	2x 50 mL
RNase A	450 µL	900 µL
Wrench	1	1
Collection Tubes	20	50

*Buffer RET and N3 contains acetic acid, wear gloves and protective eyewear when handling.
**Add 200 mL 100% ethanol to each DNA Wash Buffer before use.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important:

- **RNase A**: Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use.
- Buffer B1 precipitates below room temperature, it is critical to warm up the buffer at 37°C to dissolve the precipitates before use.
- Keep the cap tightly closed for buffer B1 after use.
- Ensure the availability of centrifuge capable of 13,000 rpm.
- Carry out all centrifugations at room temperature.

Materials supplied by users:

- 100% ethanol
- Vacuum manifold if use manifold method.
- 1.5 mL Eppendorf tubes.

EZgene ™ Plasmid Maxiprep Plunger Protocol

1. **Inoculate 150-200 mL LB containing appropriate antibiotic with 100 µL fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking.**
   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 h with vigorous shaking (~250 rpm). ***Warning:*** **Do not use more than 200 ml culture.** Need to scale up buffers if processing more than 200 mL culture.

2. **Harvest the bacterial by centrifugation at 5,000 g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.**
   Note: Complete removal of residue medium is critical for bacteria lysis in the next step.

3. **Add 10 mL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting.** Ensure that RNase A has been added into Buffer A1 before use.
   Note: Complete resuspension is critical for optimal yield.

4. **Add 10 mL Buffer B1, mix thoroughly by inverting 10 times with slightly shaking. Incubate for 5 min to obtain a slightly clear lysate.** Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.
   Attention: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37°C to dissolve before use.

5. **Add 4 mL Buffer N3, mix completely by inverting 10-15 times. Incubate at room temperature for 10 min.**

It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mixing is required to completely neutralize the solution.

6. Pour the lysate into the barrel of the filter syringe and let it set for 1 min. The white precipitates should float to the top, gently insert the plunger to expel the cleared lysate to a 50 mL tube, stop when feel strong resistance, some of the lysate may remain in the flocculent precipitate.
Note: To avoid clog of the syringe:
-Use less than 200 mL of overnight culture and mix the lysate well after adding Buffer N3.
-Spin the lysate down at 5000 rpm for 5 min and transfer the relative clear lysate to the syringe filter.

7. Add **10 mL Buffer RET. Mix well by sharp shaking for 5 times.**

8. Pull out the plunger from a DNA column and set the column in a 50 mL conical tube. Add **20 mL** lysate into the DNA column. Using the plunger, gently expel the lysate through the column. Gently pull out the plunger and process the remaining lysate as described. Discard the flowthrough.

9. Detach the end component using the plastic wrench and gently pull the plunger out. Screw the end component back to the plunger and add **20 mL DNA Wash Buffer.** Expel the Buffer out with the plunger.

10. Use the plastic wrench (Provided) to detach the end component from the maxi column and insert it into a 1.5 ml eppendorf tube.

11. Spin the column at 13,000- 15,000 rpm (Max speed) for 2 min. Decant the flow through and put the column back to the tube. Spin the column at max speed for 1 min. Transfer the column to a new eppendorf tube.

12. Add **400 µL Elution Buffer** (Pre warm the Elution Buffer at 60°C increases the yield) to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 12,000 rpm for 1 min.

13. Transfer the maxi column to a new 1.5 mL Eppendorf tube and add **400 µL Elution Buffer** to the center of the column for another elution. The first elution normally yields about 60-70% of the DNA and the second elution yields another 20-30% of the DNA.
Note: If higher DNA concentration is desired, add the eluted DNA from step 12 back to the column and spin at 12,000 rpm to elute the DNA.

Plasmid Maxiprep Vaccum Manifold Protocol

1. Inoculate 100-150 mL LB containing appropriate antibiotic with 100 µl fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking.
2. Harvest the bacterial by centrifugation at 5,000 g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.
3. **Add 10 mL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting.** Ensure that RNase A has been added into Buffer A1 before use.
4. **Add 10 mL Buffer B1, mix thoroughly by inverting 10 times with gentle shaking. Incubate for 5 -10 min to obtain a slightly clear lysate.** Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.
5. Add 4 mL Buffer N3, mix completely by inverting the tube 10 times. It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mix is required to completely neutralize the solution. Incubate at room temperature for 10 min.

6. Pour the lysate into the barrel of the filter syringe and let it set for 1 min. The white precipitates should float to the top, gently insert the plunger to expel the cleared lysate to a 50 mL tube, stop when feel strong resistance, some of the lysate may remain in the flocculent precipitate.
7. **Add 10 mL Buffer RET. Mix well by sharp shaking for 5 times.**
8. Pull out the plunger of a DNA column and insert the column to the manifold with the vacuum off. Transfer the lysate to the column and turn on the vacuum till all sample passes through the column.
9. Add **20 mL DNA Wash Buffer** to the column and allow the liquid to pass through the column. Turn off the manifold and detach the column from the manifold.
10. Use the plastic wrench (Provided) to detach the end component from the maxiprep column and insert it into a 1.5 ml eppendorf tube.
11. Spin the column at 13,000- 15,000 rpm (Max speed) for 2 min. Decant the flow through and put the column back to the tube. Spin the column at max speed for 2 min. Transfer the column to a new eppendorf tube.
12. Add 400 µL **Elution Buffer** (Prewarm the Elution Buffer at 60°C increases yield**) to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 12,000 rpm for 1 min.**
13. Transfer the column to a new eppendorf tube and add 400 µL **Elution Buffer** to the column for another elution. The first elution normally yields about 60-70% of the DNA and the second elution yields another 20-30% of the DNA.

    Note: If higher DNA concentration is desired, add the eluted DNA from step 12 back to the column and spin at 12,000 rpm to elute the DNA.

Trouble Shooting Guide

Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipeting prior adding buffer B1. • Make fresh buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N NaOH and 1%SDS).
Low Yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20ºC if the culture is not purified the same day. Do not store culture at 4ºC over night.
No DNA	Plasmid lost in Host *E.coli*	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding buffer B1.	Do not vortex or mix aggressively after adding buffer B1. Do not incubate more than 5 minutes after adding solution B1.
RNA contamination	RNase A not added to solution A1.	Add RNase A to buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Ethanol traces not completely removed from column.	Make sure that no ethanol residual remaining in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.