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Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our ezBind^{TM} matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or Elution Buffer. The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of HEK293 cells.

This kit is especially designed for purifying plasmid DNA from endA+ strains such as HB101, JM101, TG1 or their derived strains.

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	

Storage and Stability

Buffer A1 should be stored at 4 ^{o}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 production.

Kit Contents

Catalog Number	PD1711-00	PD1711-01	PD1711-02
Preps	4	50	250
ezBind™ Columns	4	50	250
Buffer A1	1.2 mL	15 mL	70 mL
Buffer B1	1.2 mL	15 mL	70 mL
Buffer N1*	1.5 mL	20 mL	90 mL
DNA Wash Buffer**	2 mL	15 mL	3 x 24 mL
Buffer KB	2.4 mL	30 mL	135 mL
EndoClean Buffer (Optional)	100 uL	1 mL (PD1214)	5 mL (PD1214)
RNase A	1.2 mg	1.5 mg	7.0 mg
Elution Buffer	0.6 mL	7.5 mL	37.5 mL

***Buffer N1 contains chaotropic salts, wear gloves and protective eyewear when handling.**

****Add 8mL（PD1711-00）60 mL (PD1711-01) or 96 mL (PD1711-02) 96-100% ethanol to DNA Wash Buffer before use. The Final ethanol is 80% (v/v)**

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.
- Add absolute ethanol as instructed to DNA Wash Buffer before use.
- Buffer B1 precipitates below room temperature, it is critical to warm up the buffer at 37 ^{o}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.
- Buffer N1 contains a chaotropic salt, wear gloves and protective eyewear when handling.

Materials supplied by users:

- 96- 100% ethanol.
- 1.5 mL microcentrifuge tubes.
- High speed microcentrifuge.
- ddH_2O.

EZgene ™ Plasmid Miniprep Protocol for endA+ Strains

1. Inoculate ***1-5 mL*** LB containing appropriate antibiotic with a fresh colony. Grow at 37 ºC for 14-16 hours with vigorous shaking.

2. Harvest the bacterial culture by centrifugation for 1 minute at 10,000 x g. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   NOTE: This protocol is optimized for E. coli strain cultured in LB medium. When using TB or 2 xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers needs to be scaled up if over amount of cultures are being processed.

3. Add ***250 µL Buffer A1*** and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).

4. Add ***250 µL Buffer B1***, mix gently by inverting 10 times (do not vortex) and incubate at room temperature for 5 minutes until the solution becomes clear.

   NOTE: Do not incubate for more than 5 minutes.

   NOTE: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 37 ºC to dissolve precipitation before use, especially in winter time.

5. Add **350 µL Buffer N1**, mix completely by inverting/shaking the vial for 5 times and vortex for 2 seconds.

   NOTE: It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; vortex for another 2 seconds.

6. Centrifuge the lysate at 13,000 rpm (14,000-18,000 g) for 10 minutes at room temperature.

7. Carefully transfer the clear lysate into a DNA column with a collection tube, avoid the precipitations, spin at 13,000 rpm for 1 minute, discard the flow-through and put the column back to the collection tube.

NOTE: If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more minutes and then transfer the clear lysate to DNA column.

8. Add ***500 µL Buffer KB*** into the spin column, centrifuge at 13,000 rpm (14,000 - 18,000 x g) for 1 minute. Remove the spin column from the tube and discard the flow-through. Put the column back to the collection tube.

9. Add ***600 µL DNA Wash Buffer*** and spin at 13,000 rpm for 1 minute. Discard the flow-through. **Repeat once.**

10. Reinsert the spin column, with the lid open, into the collection tube and centrifuge for 1 minute at 13,000 rpm.

11. Carefully transfer the spin column into a clean 1.5 mL tube and add ***50 - 100 µL sterile*** ***ddH_20*** *or* ***Elution Buffer*** （10mM Tris-HCL， PH8.5） into the column. Let it stand for 1 minute. Elute the DNA by centrifugation at 13,000 rpm (14,000-18,000 x g) for 1 minute.

12. The DNA concentration can be calculated as follows:

    DNA concentration = Absorbance 260 nm x 50 x dilution factor (µg/mL)

NOTE: The DNA is ready for downstream applications such as cloning, sequencing, RFLP or transfection of 293 cells.

NOTE: It's highly recommended to remove the endotoxin if the DNA is used for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

NOTE: Use less elution buffer if high DNA concentration is desired.

Endotoxin Removal Procedure

This protocol is designed to remove endotoxin after the plasmid DNA is purified (Buffers can be scaled up or down accordingly).

1. Add ***0.1 volume*** *of* ***EndoClean Buffer*** to the plasmid sample in a 1.5 mL sterile tube. (For example, add 0.2 mL endoClean buffer to 1 mL plasmid sample.

2. Mix by vortexing the tube a few times and put on ice for about 10 minutes until the solution is clear without turbidity. (Rocking the sample in a cold room for 10 minutes is recommended if it is available).

3. Mix well again by inverting the tube a few times.

4. Incubate the tube at 55 ºC water bath for about 5 minutes and the solution shall be turbid. Add 1/25 volume of chloroform and mix well by vortexing for 20 seconds.

5. Centrifuge at top speed at **room temperature** for 5 minutes with the brake off.

6. Carefully transfer the upper clear layer solution to 1.5 mL tube. Split in two 1.5 mL tubes, 400- 450 µL each tube.

7. Precipitate plasmid DNA with 0.1 volume of 3M NaAc (pH 5.2) and 2 volume of 100% ethanol.

   NOTE: The EndoClean-treated plasmid DNA is ready transfection of endotoxin sensitive cell lines.

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 -20 ºC if the culture is not purified the same day. Do not store culture at 4 ºC over night.
Low Yield	Low copy-number plasmid.	Increase culture volume (up to 10mL for Miniprep). Scale up the volume of buffers accordingly.
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 Buffer B1.
RNA contamination	RNase A not added to Buffer 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.