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Introduction

The E-Z 96® S.E. Plasmid DNA Isolation Kit is designed for rapid high-throughput purification of plasmid DNA from small volume of bacterial cultures. The procedure has been developed and tested using a variety of *E. coli* strains. This kit can also be used for high copy plasmid isolation .

The E-Z 96® S.E. Plasmid DNA Isolation Kit provided a fast, simple method for small scale purification of plasmids for use in routine molecular biology laboratory applications. E.Z.N.A.™ S.E. Plasmid DNA procedure is based on modified alkaline lysis of bacterial cells, followed by clearing of the lysates by filtration using a E-Z 96® lysate clearance plate, and further purification and concentration of DNA by isopropanol precipitation. The DNA obtained is dissolved with small volume of TE buffer or water and is ready to use for most downstream applications.

Benefits

The E-Z 96® S.E. Plasmid DNA Isolation Kit means:

- Speed - Plasmid DNA isolation in <60 min
- Reliability - optimized buffers guarantee pure DNA every time
- Safety - No organic extractions
- Quality - purified DNA suitable for most applications

Storage and Stability

All E-Z 96® S.E. Plasmid DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Buffer Solution I/RNase A at 4°C, all other material at 22-25°C.

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Kit Contents

Product Number	D1095-00	D1095-01	D1095-02
Purification times	1 x 96	4 x 96 Preps	20 x 96 Preps
E-Z 96 [®] Lysate Clearance Plate	1	4	20
2 mL Collection Plate	1	4	20
Solution I	35 mL	130 mL	625 mL
Solution II	35 mL	130 mL	625 mL
Neutralization Buffer	35 mL	130 mL	625 mL
RNase A	100 µL	400 µL	2.0 mL
AeraSeal Film	1	4	20
Sealing Film	5	20	100
Instruction Booklet	1	1	1

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

Supplied By Centrifuge capable of at least 5,000 x g.
User: Swinging-bucket rotor for 96-well plate
Sterile deionized water (or TE buffer)
96%-100% isopropanol
96 well 2 mL Culture Block
70% ethanol

- IMPORTANT**
1. Add vial of RNase A to bottle of Buffer Solution I and Store at 4°C.
 2. Buffer Solution II should be kept at room temperature. Check before use for SDS precipitation, and if necessary re-dissolve SDS precipitate by warming. Close Buffer Solution II bottle immediately after use to avoid the acidification of Buffer Solution II from CO₂ from air.

Note: All steps must be carried out at room temperature

E-Z 96[®] S.E. Plasmid Isolation Protocol

Procedure

Before starting, we recommend you refer to page 3 of this booklet for important information on preparation of components and required materials.

1. Culture Volume: **Innoculate 1.0-1.2 mL LB/antibiotic(s) medium placed in a 96-well 2mL culture block with *E.coli* carrying desired plasmid and grow at 37°C with agitation (180-300 rpm) for 20-24 h.**

It is strongly recommended that an *endA* negative strain of *E.coli* be used for routine plasmid isolation. Examples of such strains include DH5α[™] and JM109[™].

2. Seal the plate with tape or film and pellet bacteria by **centrifugation at 1,500-2,000 x g for 5 minutes** in a swinging-bucket rotor at room temperature.
3. **Resuspend bacterial pellet with 300 µL Solution I/RNase A. Seal the plate with Sealing film and mix by vortexing.** Complete resuspension of cell pellet is vital for obtaining good yields.
4. **Add 300 µL Solution II to each well, seal the plate with Sealing Film film, mix gently by inverting and rotating plate 10 times to obtain a cleared lysate.** Incubate at room temperature for 5 minutes. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. (Store Buffer Solution II tightly capped when not in use.)
5. **Add 300 µL Chilled (4°C) Neutralization Buffer, seal the plate with Sealing film and gently mix by inverting and rotating the plate for 15-20 times until a flocculent white precipitate forms.** Incubate on ice for 10 minutes.
6. **Optional:** Place the plate containing the cell lysate in a boiling water bath for 5 minutes. This heating step denatures and precipitate the proteins and carbohydrates that are not removed by alkaline lysis. This heating step is essential for EndA+ strains that normally have high level of endonuclease.
7. **Optional:** Place the plate on ice and incubate for 10 minutes.

■ **Clearing lysates with E-Z 96[®] Lysate Clearance plate by vacuum manifold**

- 8a. Prepare the vacuum manifold according to the instruction of the manufacturer. For Omega Vac-3 manifold, place a 2ml collection plate inside the manifold and place the top plate of manifold squarely over the base. **CAREFULLY transfer the cell lysate from step 5 or 7 into each well of a E-Z 96[®] Lysate Clearance plate.** Use a multi-channel pipet with sufficient large filling volume for the transfer. Avoid the precipitated material. Occasionally the precipitate will clog the end of the pipet tip, slightly tap the tip on the bottom of the well of the plate will break the precipitate and allow the remaining of the lysate to be transferred.
- 9a. Seal the **E-Z 96[®] Lysate Clearance plate** with **AeraSeal film** and Turn on the vacuum manifold until the lysates are completely transferred to the 2 ml Collection plate in the base.

■ **Clearing lysates with E-Z 96[®] Lysate Clearance plate by Centrifugation**

- 8b. **Place a E-Z 96[®] Lysate Clearance plate on top of a 2 mL collection plate (provided). CAREFULLY transfer the cell lysate from step 5 or 7 into each well of a E-Z 96[®] Lysate Clearance plate.** Use a multi-channel pipet with sufficient large filling volume for the transfer. Avoid the precipitated material. Occasionally the precipitate will clog the end of the pipet tip, slightly tap the tip on the bottom of the well of the plate will break the precipitate and allow the remaining of the lysate to be transferred.
- 9b. Seal the E-Z 96[®]Lysate Clearance plate with **AeraSeal** film and centrifuge at 2500 x g for 5 minutes.
10. **Add 0.7 volume of room temperature isopropanol to the samples to each sample.** (630µL isopropanol for 900µL of cell lysate). Seal the 2 mL collection plate with Sealing film and mix by inverting 3 times. Centrifuge at $\geq 5,000 \times g$ for 30 minutes at room temperature to pellet the DNA. **Carefully aspirate or decant the supernatant, making sure not to dislodge the DNA pellet.**

Note: Mark the orientation of the collection plate before centrifugation

so it can be spun at same orientation in the ethanol wash step at step 11.

11. **Wash the DNA pellet with 500 µL of 70% ethanol.** Centrifuge the 2 mL Plate (in the same orientation as before) for 15 minutes to re-concentrate the DNA pellet. Remove the solution by inverting the plate. Place inverted plate on a paper towel for 10-15 min to air dry the DNA pellet.

Note: Ensure that no alcohol droplets are visible after air drying, but do not over dry the DNA pellet because this will make the pellet difficult to redissolve.

12. **Re-dissolve the DNA pellet in 30 µL TE (10mM Tris-HCl, pH 8.5, 1 mM EDTA) or molecular grade water** by incubating overnight at room temperature. **Seal the plate with sealing film.**
13. **Yield and quality of DNA:** determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$DNA\ concentration = Absorbance_{260} \times 50 \times (Dilution\ Factor) \mu g/mL$$

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	<p>Only use LB or YT medium containing ampicillin. Do not use more than 1.2 mL</p> <p>Cells may not be dispersed adequately prior to addition of Buffer Solution II Vortex cell suspension to completely disperse.</p> <p>Increase incubation time with Buffer Solution II to obtain a clear lysate.</p> <p>Buffer Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.</p>
	Bacterial clone is not fresh.	Use fresh glycerol cultures and avoid repeated freeze/thaw cycles of clones. Always make enough replica plates and use precultures for inoculation. The remainder of the precultures can be use to set up fresh glycerol stocks.
No DNA eluted.	Lysate prepared incorrectly.	Check the stock of buffers and age of the buffers. Make sure the correct volume of buffer added to the samples.
	Cells are not resuspended completely.	Pelleted cells should be completely resuspended with Buffer Solution I. Do not add Buffer Solution II until an even cell suspension are is obtained.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of Buffer Solution II	Do not vortex or mix aggressively after adding Buffer Solution II. Adequate mixing is obtained by simply inverting and rotating tube to cover walls with viscous lysate. Reduce the culture volume if lysate is too viscous for gentle mixing.
	Culture overgrown	overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours
DNA degraded after the storage	high level of Endonuclease activity	Perform the heat inactivation step.
RNA visible on agarose gel.	RNase A not added to Buffer Solution I	Add 1 vial of RNase to each bottle of Buffer Solution I

DNA floats out of well while loading agarose gel	Ethanol not completely removed	Air dry the DNA pellet before redissolve the DNA .
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