

Contents

Introduction.	2
Kit Contents.....	3
Before Starting.....	3
E-Z 96® BACs, PACs, and P1s Isolation Protocol.....	4
E-Z 96® Plasmid Isolation Protocol.....	6
Trouble Shooting Guide.	8

Introduction

The E-Z 96® BAC/PAC DNA Isolation Kit is designed for rapid high-throughput purification of plasmid, BACs, PACs, and P1s from small volume of bacterial cultures. It is based on a modified alkaline lysis procedure which has specially adapted for spin-column and high-throughput family of procedures. The procedure has been developed and tested using a variety of low copy cosmids, BACs, PACs, P1s, and *E. coli* strains. This kit can also be used for high copy plasmid isolation. Two protocols are provided in this handbook, one for preparation of low-copy cosmid, BACs, PACs, and P1s and another protocol for high-copy plasmids and cosmids.

The E-Z 96® BAC/PAC DNA Isolation Kit provided a fast, simple method for small scale purification of BACs, PACs, P1s and plasmids for use in routine molecular biology laboratory applications. E.Z.N.A.™ BAC/PAC DNA procedure is based on modified alkaline lysis of bacterial cells, followed by clearing of the lysates by filtration using a E-Z 96® filter 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® BAC/PAC DNA Isolation Kit means:

- Speed - BACs, PACs, P1s, and 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® BAC/PAC DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Buffer T1/RNase A at 4°C, all other material at 22-25°C.

Kit Contents

Product Number	D1056-00	D1056-01	D1056-02
Purification times	1 x 96 Preps	4 x 96 Preps	24 x 96 Preps
E-Z 96 [®] Filter Plate	1	4	24
2 mL Collection Plate	1	4	24
Buffer T1	35 mL	130 mL	750 mL
Buffer T2	35 mL	130 mL	750 mL
Buffer T3	35 mL	130 mL	750 mL
Linear Acrylamide	200 µL	800 µL	4.8 mL
RNase A	100 µL	400 µL	2.5 mL
Sealing Film	6	24	150
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 User:	Centrifuge capable of at least 5,000 xg. Swinging-bucket rotor for 96-well plate Sterile deionized water (or TE buffer) 96%-100% isopropanol 10-15 mL Culture tubes 70% ethanol
IMPORTANT	<ol style="list-style-type: none"> Add vial of RNase A to bottle of Buffer T1 and Store at 4°C. Bacterial cultures for cosmids and BACs PACs and P1s, we strongly recommend using 2 x YT media for cultivation. Buffer T2 should be kept at room temperature. Check before use for SDS precipitation, and if necessary re-dissolve SDS precipitate by warming. Close Buffer T2 bottle immediately after use to avoid the acidification of Buffer T2 from CO₂ from air.

Note: All steps must be carried out at room temperature

E-Z 96[®] BACs, PACs, P1s Isolation Procedure

- Grow PAC, BAC, or P1 cultures in a 96-well or 48-well deep well plate. Fill 1.5 mL (for 96-well plate) or 2.5 mL (for 48-well plate) 2 xYT containing the appropriate selective antibiotic in an each well of the plate. Inoculate the culture with clony from plate or clones from precultures grown in a 96-well plate. **Incubate the culture at 37°C with agitation for 12-16 h with shaking at 175 rpm.**
- Pellet bacteria in the deep well plate by centrifugation for 10 minutes at 3000 x g in a centrifuge with rotor for 96-well plate.** The plate should be covered with adhesive film (not supplied) during the centrifugation. Remove the medium by inverting the plate.
- Resuspend bacterial pellet with 300 µL Buffer T1/RNase A. Seal the plate with sealing film and mix by vortexing.** Complete resuspension of cell pellet is vital for obtaining good yields.
- Add 300 µL Buffer T2 to each well seal the plate with sealing 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 T2 tightly capped when not in use.)
- Add 300 µL chilled (4°C) Buffer T3, 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.
 - **Clearing lysates with E-Z 96[®] Filter plate by vacuum manifold**
 - 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 into each well of a E-Z 96[®] Filter 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.
 - Seal the **E-Z 96[®] Filter plate** with sealing 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[®] Filter plate by Centrifugation**
 - Place a E-Z 96[®] Filter plate on top of a 2 mL collection plate (provided). CAREFULLY transfer the cell lysate from step 5 into each well of a E-Z 96[®] Filter 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.

- 7b. Seal the **E-Z 96® Filter plate** with sealing film and centrifuge at 2500 x g for 5 minutes.
8. Discard **E-Z96® Filter plate** and add 2 µL of Linear Acrylamide (supplied) to each well of the 2 mL collection plate contains cleared cell lysate. Add 0.7 volume of room temperature isopropanol to the samples. (630 µL isopropanol for 900 µL of cell lysate). Seal the 2 mL collection plate with sealing film and mix by inverting the plate 3 times. Centrifuge at $\geq 5,000$ x 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 9.

9. 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.

10. Redissolve the DNA pellet in 30 µL Buffer TE (10mM Tris-HCl, pH 8.5, 1 mM EDTA) or molecular grade water by incubating overnight at room temperature.
11. **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:

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

E-Z 96® 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 (not supplied) 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 Buffer T1/RNase A. Seal the plate with film and mix by vortexing.** Complete resuspension of cell pellet is vital for obtaining good yields.
4. **Add 300 µL Buffer T2 to each well, seal the plate with sealing 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 T2 tightly capped when not in use.)
5. **Add 300 µL Chilled (4°C) Buffer T3, seal the plate with sealing film (supplied) 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® Filter 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® Filter 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-Z96® Filter plate** with sealing 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® Filter plate by Centrifugation**

8b. **Place a E-Z 96® Filter 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® Filter 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® Filter plate with sealing film and centrifuge at 2500 xg for 5 minutes.
10. Discard **E-Z 96® Filter plate** and **add 2 µL of Linear Acrylamide (supplied) to each well of the 2 mL collection plate contains cleared cell lysate. Add 0.7 volume of room temperature isopropanol to the samples.** (630 µL isopropanol for 900 µL 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.

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:

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

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 5 mL (with high copy plasmids or 10 mL with low copy plasmids) culture with the basic protocol. Cells may not be dispersed adequately prior to addition of Buffer T2 Vortex cell suspension to completely disperse. Increase incubation time with Buffer T2 to obtain a clear lysate. Buffer T 2 if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
	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 used 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.
	Buffer T2 precipitated	Warm up the Buffer T2 to dissolve the precipitate.
	Cells are not resuspended completely.	Pelleted cells should be completely resuspended with Buffer T1. Do not add Buffer T2 until an even cell suspension is obtained.
High molecular weight DNA contamination of product.	Over mixing of cell lysate upon addition of Buffer T2	Do not vortex or mix aggressively after adding Buffer T2. 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
RNA visible on agarose gel.	RNase A not added to Buffer T1	Add 1 vial of RNase to each bottle of Buffer T2
DNA floats out of well while loading agarose gel	Ethanol not completely removed	Air dry the DNA pellet before redissolve the DNA .