

Contents

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

Introduction

The E.Z.N.A.™ BAC/PAC DNA Isolation Kit is designed for rapid high-throughput purification of 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.N.A.™ 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 spin filter column, 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.N.A.™ 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.N.A.™ BAC/PAC DNA Isolation 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	D2156-00	D2156-01	D2156-02
Purification	5	50	200
E.Z.N.A™ Filter Columns	5	50	200
2 ml Centrifuge Tubes	5	50	200
Buffer T1	5 ml	20 ml	60 ml
Buffer T2	5 ml	20 ml	60 ml
Buffer T3	5 ml	20 ml	60 ml
Glycogen (1µg/µl)	10 µl	100 µl	400µl
RNase A, Concentrate	50 µl	100 µl	400 µl
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 Microcentrifuge capable of at least 10,000 x g.

User:

Sterile 1.5 ml centrifuge tubes.

Sterile deionized water (or TE buffer)

96%-100% isopropanol

10-15 ml Culture tubes

70% ethanol

IMPORTANT

1. Add vial of RNase A to bottle of Buffer T1 provided. Store at 4°C.
2. Bacterial cultures for cosmids and BACs PACs and P1s, we strongly recommend using 2 x YT media for cultivation.
3. Buffer T2 should be kept at room temperature. Check before use for SDS precipitation, and if necessary redissolve SDS precipitate by warming. Close the 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.N.A.™ BACs, PACs, P1s Isolation Protocol

1. **Fill 5 ml 2 x YT containing the appropriate selective antibiotic in a 10-20 ml culture tubes. Inoculate the culture with clony from plate or clones from precultures grown in a 10ml tube. Incubate the culture at 37°C with agitation for 12-16 h with shaking at 175 rpm.**
2. **Pellet 1.5-5 ml bacteria in a 1.5 ml or 2 ml centrifuge tube by centrifugation at 10,000 x g for 3 min at room temperature.** Note: for pellet more than 1.5 ml culture, spin multiple times with same centrifuge tube. Discard the medium.
3. **To the bacterial pellet add 260 µl Buffer T1/RNase A. Resuspend cells completely** by vortexing. Complete resuspension of cell pellet is vital for obtaining good yields.
4. **Add 260 µl Buffer T2 and gently mix by inverting and rotating tube 5-10 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 260 µl Buffer T3 and gently mix by inverting 15-20 times until a flocculent white precipitate forms.** Incubate on ice for 5 minutes. Centrifuge at $\geq 10,000$ x g for 10 minutes at room temperature.
6. **CAREFULLY aspirate and add the clear supernatant to a E.Z.N.A.™ Filter column assembled in a 2 ml centrifuge tube (provided).** Ensure that the pellet is not disturbed and that no cellular debris is carried over into the column. Centrifuge 1 min at $\geq 10,000$ x g at room temperature to completely pass lysate through column.
7. Discard **E.Z.N.A.™ Filter column** and add 2 µl of glycogen(supplied) to the

2 ml centrifuge tube contains cleared cell lysate. Add 0.7 volume of room temperature isopropanol to the samples. (546µl isopropanol for 780µl of cell lysate). Mix the sample by vortexing for 15 seconds.

8. Centrifuge at at $\geq 10,000 \times g$ for 15 minutes at room temperature to pellet the DNA. **Carefully aspirate or decant the supernatant, making sure not to dislodge the DNA pellet.**
9. Wash the DNA pellet with 500 µl of 70% ethanol. Centrifuge the 2 ml centrifuge tube (in the same orientation as before) for 10 minutes to re-concentrate the DNA pellet. Place inverted microfuge tube 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 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:

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

Plasmid Isolation Protocol

Procedure

Before starting, we recommend you refer to page 3-4 of this booklet for important information on preparation of components and required materials.

1. **Inoculate 1.5-5 ml LB or 2 x YT medium containing appropriate antibiotic placed in a 50 ml culture flask with *E.coli* carrying desired plasmid and grow at 37°C with agitation for 12-16 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. **Pellet bacteria by centrifugation at 10,000 x g for 5 min at room temperature.**
3. **Decant or aspirate medium and discard. To the bacterial pellet add 260 µl Buffer T1/RNase A. Resuspend cells completely** by vortexing. Complete resuspension of cell pellet is vital for obtaining good yields..
4. **Add 260 µl Buffer T2 and gently mix by inverting and rotating tube 5-10 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 260 µl Buffer T3 and gently mix by inverting 15-20 times until a flocculent white precipitate forms.** Incubate on ice for 5 minutes.
6. **Optional: Place the tube 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 tube on ice and incubate for 10 minutes.**
8. Centrifuge at $\geq 10,000 \times g$ for 10 minutes at room temperature
9. **CAREFULLY aspirate and add the clear supernatant to a E.Z.N.A™ Filter column assembled in a 2 ml centrifuge tube (provided).** Ensure that the pellet is not disturbed and that no cellular debris is carried over into the column. Centrifuge 1 min at $\geq 10,000 \times g$ at room temperature to completely pass lysate through column.
10. Discard **E.Z.N.A™ Filter column** and add 2 µl of glycogen(supplied) to the 2 ml centrifuge tube contains cleared cell lysate. Add 0.7 volume of room temperature isopropanol to the samples. (546µl isopropanol for 780µl of cell lysate). Mix the sample by vortexing for 15 seconds.
11. Centrifuge at at $\geq 10,000 \times g$ for 15 minutes at room temperature to pellet

the DNA. **Carefully aspirate or decant the supernatant and discard, making sure not to dislodge the DNA pellet.**

12. Wash the DNA pellet with 500 µl of 70% ethanol. Centrifuge the 2 ml centrifuge tube (in the same orientation as before) for 10 minutes to re-concentrate the DNA pellet. Place inverted microfuge tube 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.
13. Redissolve 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.
14. **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	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 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.
	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 are 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
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 T2	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 .