## Contents


Introduction. . . . . . . . . . 2

Storage and Stability. . . . . . . . . . 2

Kit Contents. . . . . . . . . . 3

Before Starting. . . . . . . . . . 3

- Materials to Be Supplied by User. . . . . . . . . . 4

- Important Information. . . . . . . . . . 4

BACs, PACs, P1s and Plasmid Isolation Protocol (standard protocol). . . . . . . . . . 4

BACs, PACs, P1s and Plasmid Isolation Protocol (For low copy number). . . . . . . . . . 6

Troubleshooting 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 that is specially adapted for spin-column and high-throughput procedures. The procedure associated with this kit has been tested using a variety of low copy cosmids, BACs, PACs, P1s, and E.Coli strains. In addition this kit can also be used for high copy plasmid isolation. Two protocols are provided in this handbook for your convenience. The new standard protocol provide fast and reliable method for purify BAC, PAC, P1 and plasmid by using HiBind DNA spin column. The second protocol is for the isolation of low-copy cosmids, BACS, PACs, and P1s.

## New in this edition

- HiBind DNA column replace the E.Z.N.A.? Filter Column
- New standard protocol provide fast and reliable performance
- Glycogen is replaced by linear polyacrylamides
- New BAC Binding Buffer and SPM Buffer are introduced

## Storage and Stability

All E.Z.N.A.? BAC/PAC DNA Isolation Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows: Buffer T1/RNase A mixture at 4°C, and all other materials at 20°C.

## Kit Contents

| Product Number | D2156-00 | D2156-01 | D2156-02 |
|---|---|---|---|
| Purification times | 5 preps | 50 preps | 200 preps |
| HiBind? DNA MicroElute Columns | 5 | 50 | 200 |
| 2 ml Collection 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 |
| BAC Binding Buffer | 1.5 ml | 5 ml | 15 ml |
| Elution Buffer | 5 ml | 10 ml | 40 ml |
| SPM Buffer | 3 ml | 30 ml | 60 ml |
| Linear Polyacrylamides | 15 µl | 120 µl | 450 µl |
| RNase A, Concentrate | 50 µl | 100 µl | 400 µl |
| Instruction Booklet | 1 | 1 | 1 |

## Before Starting

***Prepare all components, and have the necessary materials as outlined before starting.***

**IMPORTANT**

1. Add vial of RNase A to bottle of Buffer T1 and Store at 4°C

2. **Prepare BAC Binding Buffer** as following:

| | |
|---|---|
| D2156-00 | Add 4.5 ml isopropanol (96-100%) to the bottle |
| D2156-01 | Add 15 ml isopropanol (96-100%) to the bottle |
| D2156-02 | Add 45 ml isopropanol (96-100%) to the bottle |

3. **SPM Buffer** has to be diluted with absolute ethanol (96%-100%) as following:

| | |
|---|---|
| D2156-00 | Add 7 ml ethanol (96-100%)l to the bottle |
| D2156-01 | Add 70 ml ethanol (96-100%)l to the bottle |
| D2156-02 | Add 140 ml ethanol (96-100%)l to the bottle |



## Materials Supplied by User

- Microcentrifuge capable of at least 18,000 x g (a centrifugal force of 20,000 x g corresponds to 12,000 rpm in a Beckman JA-17 rotor, or 13,000 rpm in a Sorvall SS-34 rotor).
- Sterile Deionized Water (or TE Buffer)
- Sterile 1.5 ml Centrifuge Tubes
- 10-15 ml Culture Tubes
- 70% Ethanol
- 96%-100% Isopropanol

## Important Notes

- Its strongly recommended to use 2 x YT media for the cultivation of cosmids and BACs PACs, and P1s from Bacterial Cultures.
- Buffer T2 should be kept at room temperature. Check for SDS precipitation before use. If necessary re-dissolve SDS precipitate by warming. Close the Buffer T2 bottle immediately after use to avoid acidification that may result from air $CO_2$.
- Chill Buffer T3 for BACs, PACs,P1s Isolation Protocol for precipitation enhancement.

## BACs, PACs, P1s and Plasmid Isolation Protocol (standard protocol)

1. **Isolate a single colony from a freshly streaked selective plate, and inoculate a starter culture of 2-5 ml LB or YT medium containing the appropriate selective antibiotic. Incubate for ~ 20-24 hr at 37°C with vigorous shaking (~ 300 rpm).** Use a flask with a volume at least 4 times the volume of the culture.

2. **Pellet 1.5-5 ml bacteria by centrifugation at 13,000 x g for 3 min at room temperature. Decant or aspirate medium and discard.**

3. **Resuspend the bacterial pellet by adding 200 µl of Buffer T1/RNase A solution, and vortexing.** Complete resuspension of cell pellet is vital for obtaining good yields.

4. **Add 200 µl of Buffer T2 and gently mix by inverting 5-10 times to obtain a clear lysate. Incubate at room temperature for 5 minutes.** Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. The lysate should appear viscous. Do not allow the lysis reaction to proceed more than 5 min. (Store Buffer T2 tightly capped when not in use).

5. **Add 200 µl of Chilled Buffer T3 and gently mix by inverting 15-20 times until a flocculent white precipitate forms. Incubate on ice for 5 minutes.** Do not vortex, as this will result in shearing.

6. **Centrifuge at ≥ 13,000 x g for 10 minutes at 4°C.** Promptly proceed to the next step.

7. **Carefully transfer the cleared supernatant to a new 1.5 ml tube (not supplied). Add 200µl of BAC Binding Buffer diluted with isopropanol. Invert 3-5 times to mix throughly.**
   **Note:** BAC Binding Buffer has to be diluted with isopropanol (96-100%) before use. See the label on the bottle or page 3 for detail instructions.

8. **Apply the sample to the HiBind? DNA MicroElute column assembled in a 2 ml collection tube (provided).**

9. **Centrifuge at > 8,000 x g for 30 second at room temperature. Remove the column, discard the flow-through and re-use the collection tube for next step.**

10. **Place the column back into the collection tube and add 750µl of SPM Buffer (diluted with ethanol). Centrifuge at > 8,000 x g for 30 seconds at room temperature.** Discard the flow-through and re-use the collection tube.

11. **Place the column back into the collection tube and centrifuge at maximum speed for 2 minutes to dry the column.**

12. **Place the HiBind? DNA MicroElute column into a clean 1.5 ml centrifuge tube, apply 20-50 µl of Elution Buffer (10mM Tris-HCl, pH 8.5) or water onto the center of the membrane.** Incubate 5 minutes at room temperature.



13. **Centrifuge at maximum speed for 2 minutes to elute the DNA.**

14. **Store the eluted DNA at -20°C.**

## BACs, PACs, P1s and Plasmid Isolation Protocol (For low copy number)

1. **Isolate a single colony from a freshly streaked selective plate, and inoculate a starter culture of 2-5 ml LB or YT medium containing the appropriate selective antibiotic. Incubate for ~ 20-24 hr at 37°C with vigorous shaking (~ 300 rpm).** Use a flask with a volume at least 4 times the volume of the culture.

2. **Pellet 1.5-5 ml bacteria by centrifugation at ≥13,000 x g for 3 min at room temperature. Decant or aspirate medium and discard.**

3. **Resuspend the bacterial pellet by adding 260 µl of Buffer T1/RNase A solution, and vortexing.** Complete resuspension of cell pellet is vital for obtaining good yields.

4. **Add 260 µl of Buffer T2 and gently mix by inverting 5-10 times to obtain a clear lysate. Incubate at room temperature for 5 minutes.** Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. The lysate should appear viscous. Do not allow the lysis reaction to proceed more than 5 min. (Store Buffer T2 tightly capped when not in use).

5. **Add 260 µl of Chilled Buffer T3 and gently mix by inverting 15-20 times until a flocculent white precipitate forms. Incubate on ice for 5 minutes.** Do not vortex, as this will result in shearing.

6. **Centrifuge at ≥ 14,000 x g for 10 minutes at 4°C.** Promptly proceed to the next step.

7. **Carefully transfer the cleared supernatant to a new 1.5 ml tube (not supplied). Add a 0.7 volume of room temperature isopropanol and 2 µl of the supplied linear polyacrylamides to the samples**( 546 µl of isopropanol for 780 µl of cell lysate). **Mix the sample by vortexing for 15 seconds.**

8. **Centrifuge at ≥ 14,000 x g for 10 minutes at room temperature to pellet the DNA. Carefully aspirate or decant the supernatant and discard, making sure not to dislodge the DNA pellet.**

9. **Wash the DNA pellet with 500 µl of 70% ethanol. Centrifuge the 2 ml collection tube containing pellet** (in the same orientation as before) **for 10 minutes. Carefully aspirate or decant the supernatant, being careful not to dislodge the DNA pellet. Invert the tube containing the DNA pellet 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, with out overdrying the DNA pellet. Overdrying of the DNA pellet will make redissolving the pellet difficult.

10. **Redissolve the DNA pellet by adding 30-50 µl of Elution Buffer (10 mM Tris-HCl, pH 8.5), and incubating overnight at room temperature**

11. **Yield and quality of DNA: Determine the absorbance of an appropriate dilution of the sample at 260 nm and then at 280 nm.** The DNA concentration is calculated as follows: DNA concentration = $A_{260}$ x 50 x (Dilution Factor) µg/ml. A ratio of ($A_{260}$) / ($A_{280}$) is an indication of nucleic acid purity. Alternatively, yield (as well as quality) can sometimes be best determined by agarose gel/ethidium bromide electrophoresis.



# Troubleshooting Guide

*Please use this guide to solve any problems that may arise. We hope that it will aid in clearing up any questions for you, if for any reason you need further assistance the scientists at Omega Bio-Tek, Inc. are always happy to answer any questions you may have about either the information and procedures of this manual or molecular biology applications (see page 12 for contact information).*

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**Low DNA yields**

| | Problem | Suggestion |
|---|---|---|
| a) | 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 have been dispersed adequately prior to the addition of Buffer T2. Make sure to vortex cell suspension to completely disperse. |
| | | Increase incubation time with Buffer T2 to obtain a clear lysate. |
| | | Buffer T2 if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS. |
| b) | Bacterial Clone is not fresh. | Use fresh glycerol cultures and avoid repeated freezing/thawing cycles of clones. Always make enough replica plates and use precultures for inoculation. Any remaining precultures can be used to set up fresh glycerol stocks. |

**No DNA Eluted**

| | Problem | Suggestion |
|---|---|---|
| c) | Lysate prepared incorrectly. | Check the stock of buffers and age of the buffers. Make sure that the correct volume of buffer has been added to the samples. |
| d) | Buffer T2 precipitated | Warm up the Buffer T2 to dissolve the precipitate. |
| e) | Cells are not completely resuspended | Pelleted cells should be completely resuspended with Buffer T1.Do not add Buffer T2 until an even cell suspension is obtained. |

**Possible Problems and Suggestions**

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**High molecular weight DNA contamination of product.**

| | Problem | Solution |
|---|---|---|
| f) | Over mixing of cell lysate upon addition of T2 | Do not vortex or mix aggressively after adding Buffer T2. |
| g) | Culture overgrown | Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours. |

**DNA degraded after the storage**

| | Problem | Solution |
|---|---|---|
| h) | High levels of endonuclease activity. | Perform the heat inactivation step. |

**RNA visible on agarose gel**

| | Problem | Solution |
|---|---|---|
| I) | RNase A not added to Buffer T2. | Add 1 vial of RNase to each bottle of Buffer T2. |
| j) | DNA floats out of well while loading agarose gel. | Air dry the DNA pellet before redissolving the DNA. |

Please Call, Fax , or e-mail us to place an order.

We will be happy to answer any questions for you.

Tel: 770-931-8400 (US) Fax: 770-931-0230 (US) e-mail: info@omegabiotek.com

Tel: 1-800-832-8896 (Toll free) Fax: 1-888-624-1688 (Toll free)

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