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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-Tek's proprietary HiBind® matrix that avidly, but reversibly, binds DNA or RNA under certain optimal conditions, allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The E.Z.N.A.™ BAC/PAC Maxi Kit combines the power of HiBind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high quality BAC/PAC DNA. Omega Bio-Tek's Maxi Spin columns facilitate the binding, washing, and elution steps, thus enabling multiple samples to be simultaneously processed.

Yields vary according to copy number, *E.coli* strain, and conditions of growth, typically, 200 ml of overnight culture in an 2x YT medium typically produces 20-50 µg of BAC DNA. Isolated BAC, PAC DNA are suitable for automated fluorescent DNA sequencing, restriction enzyme digestion and other manipulations.

Storage and Stability

All E.Z.N.A.™ BAC/PAC Maxi kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: T I/RNase A at 4ºC, all other material at 22-25ºC.

Kit Contents

Product Number	D2154-00	D2154-01	D2154-02
Purification times	2 Preps	5 Preps	20 Preps
HiBind® BAC Maxi Columns	2	5	20
50 ml collection tubes	2	5	20
Buffer T1	50 ml	120 ml	2 x 250 ml
Buffer T2	50 ml	120 ml	2 x 250 ml
Buffer T3	50 ml	120 ml	2 x 250 ml
BAC Binding Buffer	15 ml	30 ml	2 x 75 ml
SPW Wash Buffer	20 ml	50 ml	2 x 50 ml
RNase A	300 µl	0.7ml	2 x 1.2 ml
Elution Buffer	10 ml	60 ml	120 ml
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:

- High speed centrifuge capable of 12,000 × g
- Centrifuge with swing-bucket rotor capable of at least 3000 x g (Optional for centrifugation Protocol)
- Nuclease-free 50 ml centrifuge tubes (Falcon® tubes recommended)
- Vacuum manifold (Optional for Vacuum Protocol)
- Ice
- 50 ml centrifuge tube
- High speed centrifuge tubes (polycarbonate or Corex®)
- Sterile deionized water (or TE buffer)
- Isopropanol
- Absolute (95%-100%) ethanol

IMPORTANT

1. Add RNase A to bottle of Buffer T1 provided. Store at4°C.
2. BAC Binding Buffer must to be diluted with **isopropanol** before use:

   D2154-00 Add 35 ml isopropanol

   D2154-01 Add 70 ml isopropanol

   D2154-02 Add 175 ml isopropanol
3. Add **absolute ethanol (96-100%)** to SPW Wash Buffer as follow:

   D2154-00 Add 80 ml absolute ethanol

   D2154-01 Add 200 ml absolute ethanol

   D2154-02 Add 200 ml absolute ethanol per bottle

3. Optional: Pre-heat Elution Buffer to 70°C.

Store diluted SPW and BAC Binding Buffer at room temperature

Protocol 1: E.Z.N.A.™ BAC/PAC Vacuum/Spin Protocol

This Protocol is designed to isolate 20-100 µg of BAC DNA from up to 200 ml overnight cultures using Omega's E.Z.N.A.™ BAC/PAC Maxi Kit.

- **Growth of Bacterial Culture**

1. **Culture volume: Inoculate 100-200 ml 2 x YT medium contains appropriate antibiotic placed in a 200-400 milliliter culture flask with *E.coli* carrying desired BAC or PAC and grow at 37°C with agitation for 16-20 h. For best results use overnight culture as the inoculum.**

   Optimal growth conditions for bacteria are vital in obtaining maximal BAC DNA yields. To achieve the best conditions, pick a single isolated colony from a freshly transformed or freshly plated bacteria to inoculate a 2-5ml starter culture containing the appropriate antibiotic. Then, incubate for ~8hr at 37°C with vigorous shaking (~300rpm). Next, use to inoculate an appropriate volume of pre-warmed liquid growth medium containing desired antibiotic. Grow at 37°C for 16-20 hr with vigorous shaking(~300rpm).(use a flask or vessel with a volume of at least 3-4 times the volume of the culture and dilute the starter culture 1/500 to 1/1000 into growth medium).

   If you are using a frozen glycerol stock as the inoculun, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then pick out a single colony and inoculate the 2-5ml starter culture as described above.

- **Lysis of Bacterial Cells With Alkaline-SDS Solution**

2. **Pellet 100-200 ml bacteria in appropriate vessels by centrifugation at 3,500-5,000 × g for 10-15 min at room temperature.**

3. **Decant or aspirate medium and discard. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel. To the bacterial pellet add 20 ml Buffer T1/RNase A.** Resuspend cells completely by vortexing or pipetting up and down. *Complete resuspension of cell pellet is vital for obtaining good yield.*

4. **Add 20 ml Buffer T2 and gently mix the cell lysate by inverting the tube for 10-20 times. Incubate 3 minutes at room temperature.** Avoid vigorous mixing as this will shear chromosomal DNA and lower BAC 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 20 ml Cold Buffer T3 to the sample, mix immediately and throughly by inverting tube 20 times or until a flocculent white precipitate forms.** Incubate on ice for 10 minutes. Centrifuge at 15,000 x g for 15 minutes at 4°C .

   Note: The buffers must be mixed throughly. If the mixture appears to still be viscous, brownish and conglobated, more mixing will be required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields. Increasing centrifugation speed is helpful to completely remove the precipitated bacterial cell material. A tightly packed cell debris pellet indicates efficient lysis.

   Note: For faster removal of the precipitated bacterial cell material, one may order Omega's Lysate Clearance Filter Syringes to replace centrifugation step. This filter cartridge completely removes SDS precipitates and clears bacterial lysates using a filter syringe.

6. **Transfer cleared supernatant to an appropriate vessel. Add 20 ml BAC Binding Buffer, mix immediately and throughly by inverting tube 20 times. Incubate at room temperature for 2-5 minutes.**

   Note: The BAC Binding Buffer must diluted with isopropanol before use. See instruction on page 3 or instruction from bottle label.

- **BAC/PAC DNA Purification Using HiBind® BAC Maxi Column**

7. **Set up vacuum manifold. Carefully transfer 20 ml of the clear supernatant into a new HiBind® BAC Maxi Column assembled onto vacuum manifold, making sure that no cellular debris is carried over**. The Maxi column has a maximal capacity of 23 ml. Apply the vacuum to draw the liquid through the membrane of the HiBind Maxi column. Turn off the vacuum when all the liquid pass through the membrane. Add the remaining cleared lysate to the column and apply the vacuum until all the liquid pass through the column. Turn off the vacuum.

8. **Add 20 ml of SPW Wash Buffer to the HiBind BAC Maxi column. Turn on the**

vacuum until all liquid pass through the column. After all liquid pass through the membrane, continue the vacuum for additional 15 minutes to further dry the column.

Note: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. See labels for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

- **BAC DNA Elution From HiBind® BAC Maxi Column**

9. **Place column into a clean 50 ml centrifuge tube. Add 0.7-2.0 ml (depending on desired concentration of the final product) of Elution Buffer (or TE buffer) directly onto the column matrix**. Allow column to sit 5-10 min at room temperature. Centrifuge at a maximum speed (no more than 5,000 x g) for 5 min to elute DNA. This represents approximately 50-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Also, pre-heating the water to 70°C prior to elution may significantly increase yields.

Protocol 2: E.Z.N.A.™ BAC/PAC DNA Spin Protocol

This Protocol is designed to isolate 20-100 µg of BAC DNA from up to 200 ml overnight cultures using Omega's E.Z.N.A.™ BAC/PAC Maxi Kit.

- **Growth of Bacterial Culture**

1. **Culture volume: Inoculate 100-200 ml 2 x YT medium contains appropriate antibiotic placed in a 200-400 milliliter culture flask with *E.coli* carrying desired BAC or PAC and grow at 37°C with agitation for 16-20 h. For best results use overnight culture as the inoculum.**

   Optimal growth conditions for bacteria are vital in obtaining maximal BAC DNA yields. To achieve the best conditions, pick a single isolated colony from a freshly transformed or freshly plated bacteria to inoculate a 2-5ml starter culture containing the appropriate antibiotic. Then, incubate for ~8hr at 37°C with vigorous shaking (~300rpm). Next, use to inoculate an appropriate volume of pre-warmed liquid growth medium containing desired antibiotic. Grow at 37°C for 16-20 hr with vigorous shaking(~300rpm).(use a flask or vessel with a volume of at least 3-4 times the volume of the culture and dilute the starter culture 1/500 to 1/1000 into growth medium)

   If you are using a frozen glycerol stock as the inoculun, streak it onto an agar plate containing the appropriate antibiotic for single colony isolation. Then pick out a single colony and inoculate the 2-5ml starter culture as described above.

- **Lysis of Bacterial Cells With Alkaline-SDS Solution**

2. Pellet 100-200 ml bacteria in appropriate vessels by centrifugation at 3,500-5,000 × g for 10-15 min at room temperature.

3. **Decant or aspirate medium and discard. To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel. To the bacterial pellet add 20 ml Buffer T1/RNase A.** Resuspend cells completely by vortexing or pipetting up and down. *Complete resuspension of cell pellet is vital for obtaining good yield.*

4. **Add 20 ml Buffer T2 and gently mix the cell lysate by inverting the tube for 10-20 times.** Incubate 3 minutes at room temperature. Avoid vigorous mixing as

this will shear chromosomal DNA and lower BAC 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 20 ml Cold Buffer T3 to the sample, mix immediately and throughly by inverting tube 20 times or until a flocculent white precipitate forms.** Incubate on ice for 10 minutes. Centrifuge at 10,000 x g for 15 minutes at 4°C .

   Note: The buffers must be mixed throughly. If the mixture appears to still be viscous, brownish and conglobated, more mixing will be required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields. Increasing centrifugation speed is helpful to completely remove the precipitated bacterial cell material. A tightly packed cell debris pellet indicates efficient lysis.

   Note: For faster removal of the precipitated bacterial cell material, one may order Omega's Lysate Clearance Filter Syringes to replace centrifugation step. This filter cartridge completely removes SDS precipitates and clears bacterial lysates using a filter syringe.

6. **Transfer cleared supernatant to an appropriate vessel. Add 20 ml BAC Binding Buffer, mix immediately and throughly by inverting tube 20 times. Incubate at room temperature for 2-5 minutes.**

   Note: The BAC Binding Buffer must diluted with isopropanol before use. See instruction on page 3 or instruction from bottle label.

- **BAC/PAC DNA Purification Using HiBind® BAC Maxi Column**

7. **Carefully transfer 20 ml of the clear supernatant to a clean HiBind® BAC Maxi Column assembled in an 50 ml collection tube, making sure that no cellular debris is carried over**. The Maxi column has a maximal capacity of 23 ml. Centrifuge at 3000 × g for 5 minutes at room temperature to pass the cleared lysate through the column. Discard the flow-through liquid and add the remaining cleared lysate to the column. Centrifuge as above and repeat until the entire sample has been passed through. Discard the flow-through and reuse the collection tube in Step 8.

8. **Wash the column again by adding 20 ml of SPW Wash Buffer diluted with ethanol. Centrifuge as above and discard flow-through and re-use the collection tube.**

   Note: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. See labels for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

9. **Centrifuge the empty capped column for 10 min at a maximum speed (no more than 5,000 x g) to dry the column matrix.** Remove any traces of ethanol from the column's inner surface or O-ring using a pipette.

   DO NOT skip this step - it is critical for removing traces of ethanol that may otherwise interfere with downstream applications.

- **BAC DNA Elution From HiBind® BAC Maxi Column**

10. **Place column into a clean 50 ml centrifuge tube. Add 0.7-2.0 ml (depending on desired concentration of the final product) of Elution Buffer (or TE buffer) directly onto the column matrix**. Allow column to sit 5-10 min at room temperature. Centrifuge at a maximum speed (no more than 5,000 x g) for 5 min to elute DNA. This represents approximately 50-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Also, pre-heating the water to 70°C prior to elution may significantly increase yields.

Short Protocol For Experienced Users

1. Pellet cells 100-200 ml overnight culture.
2. Resuspend cells in 20 ml Buffer T1/RNase A.
3. Add 20 ml Buffer T2. Mix gently but throughly by inverting 10-20 times to obtain cleared lysate. A brief incubation at RT may be required.
4. Add Cold 20 ml Buffer T3 and mix well to form white precipitate.
5. Centrifuge at a maximal (at least 8,000 x g) speed for 10 min at 4°C. Transfer the cleared supernatant and add 20 ml BAC Binding Buffer. Incubate for 2-5 minutes.
6. Transfer cleared lysate in to a HiBind® BAC Maxi column placed in a 50 ml collection tube. Centrifuge or vacuum to pass the liquid through the membrane.
7. Wash column with 20 ml SPW Wash Buffer.
8. Dry the column
9. Elute BAC with 0.7-2.0 ml Elution Buffer or TE buffer. And Centrifuge at maximal speed for 2 min.
10. Concentrate the eluted BAC DNA with isopropanol
11. Dissolve the BAC DNA with elution Buffer.

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 200 ml with high copy BACs. Cells may not be dispersed adequately prior to addition of Solution II. Vortex cell suspension to completely disperse. Increase incubation time with Solution II to obtain a clear lysate.
	Bacterial culture overgrown or not fresh.	Do not incubate cultures for more than 16 hr at 37°C. Storage of cultures for extended periods prior toBAC DNA isolation is detrimental.
No DNA eluted.	DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPM Wash Buffer Concentrate as instructed on the label.
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.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase A_{260}.	Make sure to wash column as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
RNA visible on agarose gel.	RNase A not added to Buffer T1	Add 1 vial of RNase to each bottle of Solution I.
BAC DNA floats out of well while loading agarose gel	Ethanol not completely removed from column following wash steps.	Centrifuge column as instructed in Step 11 to dry .
BAC DNA will not perform in downstream application	Traces of ethanol remain on column prior to elution.	The column must be washed with absolute ethanol (Step 10) and dried before elution. Ethanol precipitation may be required following elution
A260/A280 ratio is high or low	Wash Buffer is diluted with ethanol contains impurities.	Check the absorbency of the ethanol between 230-280nm. Do not use the ethanol with high absorbency
	Purified BAC has RNA contamination	Ensure that Rnase A is added to the solution I before use.
	Background reading is high due to the silica fines	Centrifuge the purified DNA for 1-2 minutes and transfer the sample to a new tube, measure the OD again.