

BAC DNA MiniPrep Kit

Product # 18000

Product Insert

The BAC DNA MiniPrep Kit is designed for the rapid preparation of BACs (bacterial artificial chromosomes) and other large DNA constructs from small batch cultures of *Escherichia coli*. Purification of constructs up to 130 kb in size has been verified. The DNA is preferentially purified from other cellular components such as genomic DNA and RNA without the use of ethanol or other alcohols, a significant improvement over other techniques to achieve full compatibility with alcohol sensitive applications. Typical DNA recoveries range between 0.6 and 1 µg from 3.0 mL of bacterial culture. The purified DNA is fully digestible with all restriction enzymes tested, and is completely compatible with manual or automated sequencing to achieve 95-100% accuracy.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds DNA under high salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The process for the isolation of plasmid DNA involves first pelleting 3 mL of an overnight culture of *E. coli* harbouring the BAC or plasmid of interest using centrifugation (please see the flow chart on page 3). The pellet is then resuspended in the provided Resuspension Buffer, which contains RNAase A. Lysis Solution is then added to the sample in order to assist in the lysis of the bacterial cells. Next, Neutralization Solution is added to the sample which will neutralize the sample and cause precipitation of the proteins and genomic DNA that is present. The resulting suspension is spun down, and the clarified lysate containing the plasmid DNA is then collected. RNase T1 is added then added to the lysate and it is incubated at 50°C for 30 minutes. Binding Solution is then added to the lysate, and it is applied to a provided column through centrifugation. Norgen's resin binds DNA in a manner that depends on ionic concentrations, thus the DNA will bind to the column while most of the RNA, proteins and other contaminants will either flowthrough or be retained on top of the resin. The bound DNA is then washed using the provided Wash Solution in order to remove any remaining impurities, and the purified plasmid DNA is eluted with the Elution Buffer.

Specifications

Kit Specifications	
Column Binding Capacity	25 µg
Size of Plasmids Purified	Up to 130 kbp
Average Yield from 3 mL of Culture	0.6 – 1 µg
Time to Complete 10 Purifications	1 hour

Advantages

- Fast and easy processing using a rapid spin-column format
- Alcohol-free protocol – thus the DNA is compatible with alcohol sensitive downstream applications
- High yield of plasmid DNA – up to 1 µg from 3 mL of culture

Kit Components

Component	Product # 18000 (25 preps)
Resuspension Buffer	6 mL
Lysis Solution	6 mL
Neutralization Solution	6 mL
Binding Solution	2 mL
Wash Solution	22.5 mL
Elution Buffer	3 mL
RNAse A	8500 units
RNAse T1	18000 units
Micro Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
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Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The **RNAse A** and **RNAse T1** should be stored at -20°C upon arrival. Once RNAse A has been added to the **Resuspension Buffer**, however, the solution should be stored at 4°C. All the reagents should remain stable for at least 1 year in their unopened containers.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

The **Binding Solution** and **Wash Solution** contain guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

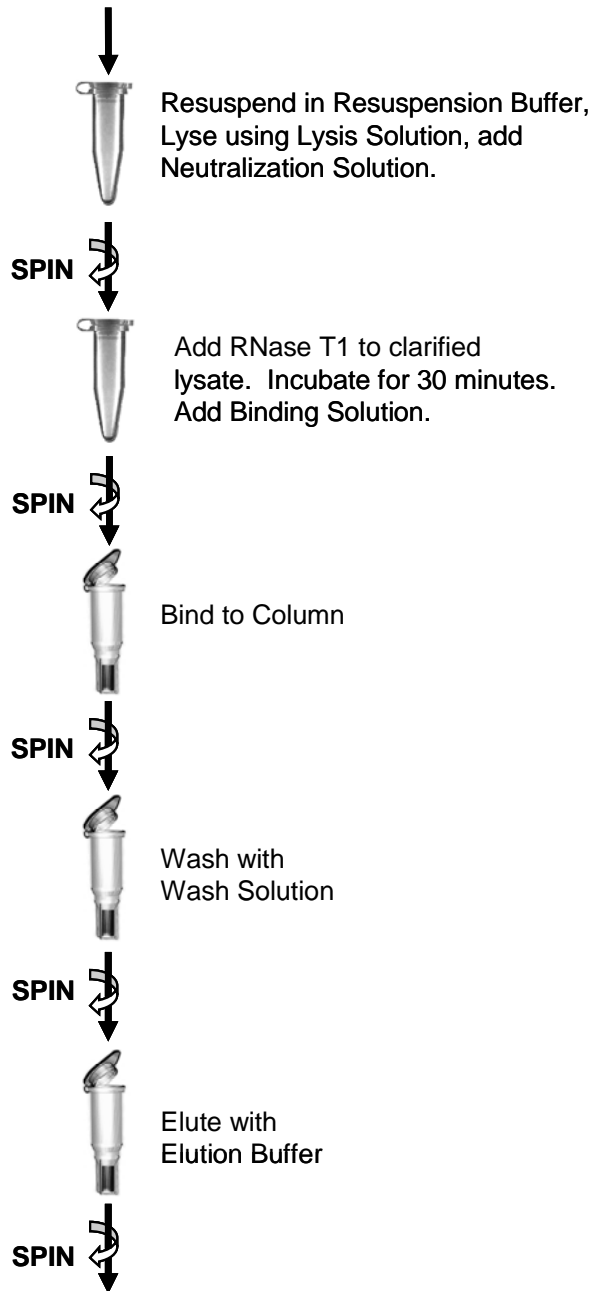
Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 50°C incubator

Flow Chart

Procedure for the Rapid Preparation of BAC DNA

Pellet overnight bacterial culture containing BAC or plasmid of interest



Pure BAC or Plasmid DNA

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g except where noted. Please check your microcentrifuge specifications to ensure proper speed. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes prior to use:

- Ensure that all solutions, except the **Resuspension Buffer**, are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- Take the entire amount of **RNAse A** and add it to the **Resuspension Buffer**. The label on the bottle has a box that can be checked to indicate that the RNAse A has been added. The solution can be stored for up to 6 months at 4°C.
- Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure.

1. Lysate Preparation

- a. Transfer 1.5 mL of bacterial culture to a microcentrifuge tube and centrifuge for 20 seconds to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.
- b. Add an additional 1.5 mL of bacterial culture to the tube and centrifuge for 20 seconds. Pour off the supernatant carefully as in step 1a.
- c. Add 200 µL of **Resuspension Buffer** (containing **RNAse A**; see Notes Prior to Use) to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing. Incubate at room temperature for 5 minutes.
- d. Add 200 µL of **Lysis Solution** to the cell suspension, cap the tube, and mix the contents by gently inverting the tube several times. Do not vortex as this will shear the genomic DNA. The suspension should become clear and viscous as the cells begin to lyse. Continue mixing until a clear lysate is obtained.

Note: If necessary, allow the solution to incubate at room temperature provided the total incubation time is no more than 5 minutes. This step is also critical for the denaturation of cellular proteins and genomic DNA.

- e. Add 200 µL of **Neutralization Solution** and immediately mix by inverting the tube several times. The solution will become turbid as insoluble particles from denatured materials start to form.
- f. Centrifuge for 10 minutes to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.
- g. Transfer the clarified lysate to a fresh microcentrifuge tube. Ensure that none of the white precipitate is transferred.

- h. Add the appropriate amount of RNase T1 specified on the label to the clarified lysate, cap the collection tube and mix gently. Incubate the lysate at 50°C for 30 minutes.

2. Binding to Column

- a. Add 60 μ L of **Binding Solution** to the lysate and mix well
- b. Assemble a spin column with a provided collection tube. Transfer the lysate into the micro spin column, cap the column and then centrifuge the unit for 1 minute.
- c. After centrifugation, separate the column from its collection tube. Discard the flowthrough and reassemble the spin column with its collection tube.

3. Washing Bound DNA

- a. Apply 600 μ L of **Wash Solution** to the column, and centrifuge for 2 minutes.
- b. Ensure that all the **Wash Solution** has passed through the column and that the column is dry. Spin for an additional minute if necessary.
- c. Discard the flowthrough and reassemble the column with a provided **Elution tube**.

4. Elution of Clean DNA

- a. Add 30 - 50 μ L of **Elution Buffer** to the column and centrifuge for 2 minutes at 2,000 x g. Centrifuge the elution for an additional 1 minute at **14,000 x g**.

For more concentrated DNA use a 30 μ L elution volume. If higher recoveries are required, use a 50 μ L elution volume.

Note: If the speed of the centrifuge can not be adjusted, the elution of DNA can also be performed at 14,000 x g for one minute. Lower recoveries of DNA may be expected.

- b. (Optional): An additional elution can be performed by repeating step **4a**. This elution should be collected into a separate tube to avoid diluting the DNA solution in the first elution.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Plasmid did not propagate	Ensure that the appropriate growth medium, supplements and antibiotics were used for the host cell and plasmid of interest.
	Inoculum cell culture was old	Old bacterial cells are a poor source of plasmid DNA. Bacterial cell inoculum should be prepared from fresh single colonies, grown in a test-tube overnight and immediately used for inoculum preparation. Prolonged incubation or storage of culture in the fridge almost guarantees poor results.
	Insufficient lysis of cells	The Lysis Solution may have formed precipitates. Warm and mix gently before use.
	Cell resuspension was incomplete	Pelleted cells should be completely resuspended in the Resuspension Buffer . Do not add Lysis Solution until a homogeneous suspension is obtained.
	Proper Elution Buffer was not used	The provided Elution Buffer has been optimized for high elution recoveries. If water is used, ensure that the pH is between 7 and 8.
DNA does not perform well in downstream applications	DNA was not washed with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed with Wash Solution . Salt may interfere with downstream applications, and thus must be washed from the column.
	A different Elution buffer was used	If a different Elution buffer other than the one provided in the kit was used, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

Related Products	Product #
Plasmid MiniPrep Kit	13300
Plasmid MaxiPrep Kit	15300
Low Copy Plasmid MiniPrep Kit	17800

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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