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BAC DNA MiniPrep Kit — Product Insert

Product # 18050

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. 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

BAC DNA could be purified with this kit using either an alcohol-precipitation protocol or a column-based protocol, depending on the volume of culture input and/or downstream application requirement. The process for the isolation of plasmid DNA involves first pelleting 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. 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. From this point on, the user could choose between the precipitation or column procedure. **For all input culture volumes (up to 100 mL) and downstream applications that require intact plasmid**, the alcohol precipitation protocol is to be followed. Isopropanol is added to the lysate and the DNA is precipitated by centrifugation. The resulting DNA pellet is resuspended and treated with RNase, followed by a second ethanol precipitation. The final DNA pellet is resuspended in the provided TE. **For input culture volumes of up to 5 mL and downstream applications that are sensitive to contaminants from purification (such as PCR and sequencing)**, an alternative column purification protocol could be followed. RNase is added to the lysate. Binding Solution and ethanol 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.

This kit is designed to process either:

- **50 x 1.5 mL culture samples**
- **30 x 5 mL culture samples**
- **15 x 10 mL culture samples**
- **6 x 40 mL culture samples**
- **3 x 100 mL culture samples.**

Specifications

Kit Specifications	
Input Culture Volume	**Alcohol Precipitation**: Up to 100 mL **Column Purification**: Up to 5 mL
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 alcohol precipitation or a rapid spin-column format
- High yield of plasmid DNA – up to 1 µg from 3 mL of culture
- Versatile protocol that allows different volume of input to be processed

Kit Components

Component	Product # 18050
Resuspension Buffer	20 mL
Lysis Solution	40 mL
Neutralization Solution	30 mL
Binding Solution	15 mL
Wash Solution	22 mL
Elution Buffer	15 mL
TE Buffer	60 mL
RNAse	250 µL
Spin Columns inserted in Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. The **RNAse** should be stored at -20°C upon arrival. 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*.

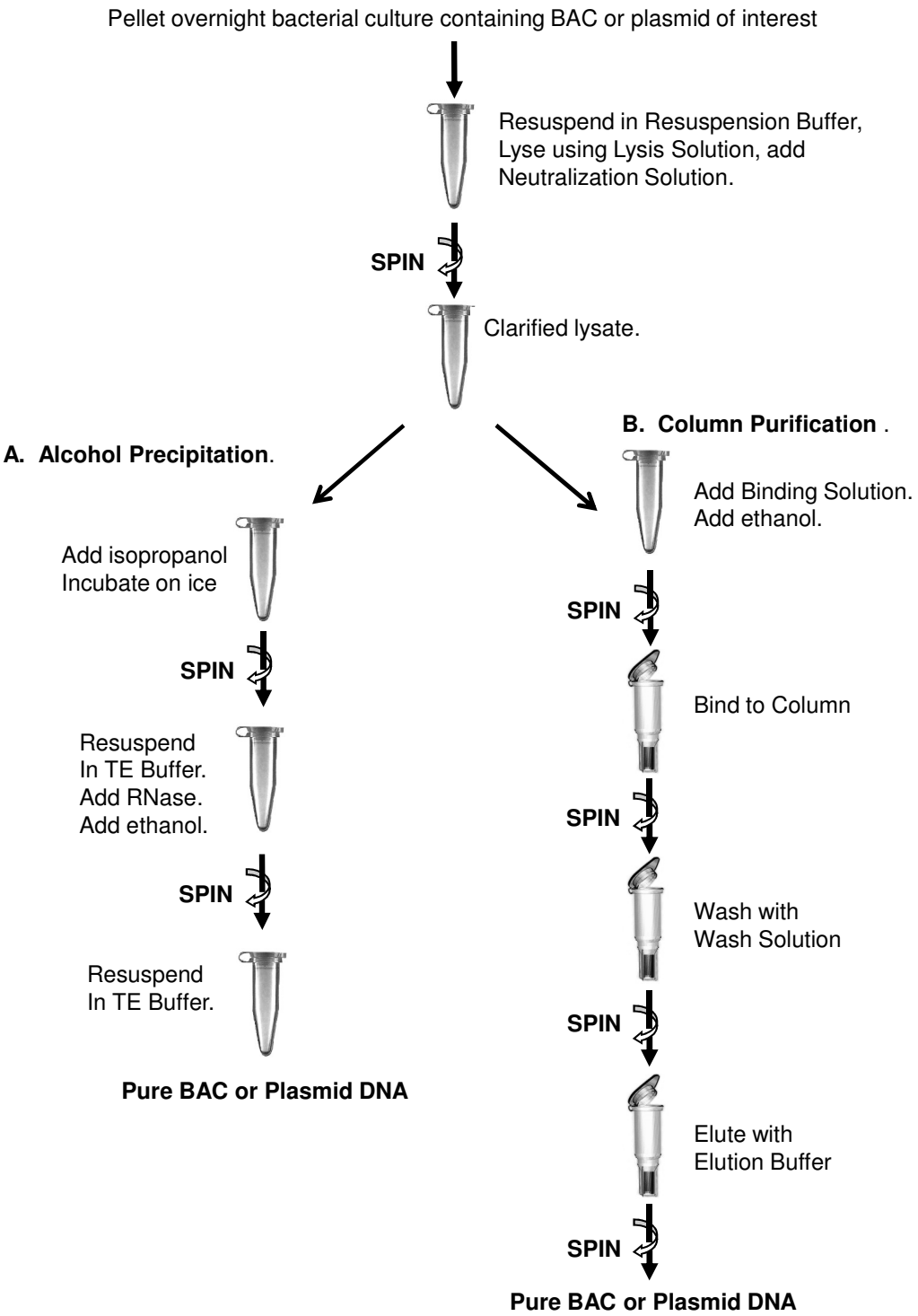
The **Binding Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge (with refrigeration)
- Centrifuge with a swinging bucket rotor capable of 3000 x g (with refrigeration)
- Centrifuge capable of 14 000 x g (with refrigeration)
- 1.5 mL microcentrifuge tubes
- 50 mL conical tubes
- Centrifugation Bottles
- 37°C incubator
- Isopropanol (Alcohol Precipitation Protocol)
- 96 – 100% Ethanol (All Protocols)

Flow Chart

Procedure for the Rapid Preparation of BAC DNA



Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 *x g* (~ 14,000 RPM) 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:

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Chill **Resuspension Buffer**, **Neutralization Solution** and Isopropanol on ice prior to the start of the procedure
- Ensure that the remaining solutions are at room temperature, and that no precipitates have formed. If necessary, warm the solutions and mix until the solutions become clear.
- For **Column Purification**, prepare a working concentration of the **Wash Solution** by adding 50 mL of 96 – 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Bacterial cultures grown overnight at 37°C in LB medium are optimal for this procedure.
- The protocol is provided for isolating BAC or plasmid from different volumes of input culture. Please follow **Table 1** below for the amount of solution required for each step of the lysate preparation.
- The lysate preparation steps are common for all procedures (Section 1). For BAC or plasmid purification, please follow **Section 2 for Alcohol Precipitation** or **Section 3 for Column Purification.**

Section 1. Lysate Preparation

1. Lysate Preparation

a. Transfer the bacterial culture to an appropriate centrifugation vessel (either a microcentrifuge tube, conical tube or centrifugation bottle). Centrifuge to pellet the cells (14,000 x g in a benchtop microcentrifuge for 5 minutes for up to 1.5 mL culture or 3,000 x g in a swing-bucket centrifuge for 10 minutes for larger volumes up to 100 mL). Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

b. Add an appropriate amount of cold **Resuspension Buffer** based on recommendation in **Table 1** to the cell pellet. Resuspend the cells by pipetting in and out, or by gentle vortexing.

Table 1. Amount of Solutions Required for Lysate Preparation

Input Culture	1.5 mL	5 mL	10 mL	40 mL	100 mL
Resuspension Buffer	200 µL	0.5 mL	1 mL	4 mL	10 mL
Lysis Solution	400 µL	1 mL	2 mL	8 mL	20 mL
Neutralization Solution	300 µL	0.75 mL	1.5 mL	6 mL	15 mL

c. Add an appropriate amount of **Lysis Solution** according to **Table 1** 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.

d. Add an appropriate amount of cold **Neutralization Solution** according to **Table 1** and immediately mix by inverting the tube several times. The solution will become turbid as insoluble particles from denatured materials start to form. Incubate on ice for 10 minutes.

e. Centrifuge the lysate for 10 minutes at 4°C at ≥ 14,000 x g in the appropriate centrifuge to clarify the lysate. An insoluble pellicle will be collected on the bottom of the centrifuge tube.

f. Transfer the clarified lysate to a fresh centrifugation vessel (same size as **Step 1a**). Ensure that none of the white precipitate is transferred.

g. Proceed to either **Section 2** for alcohol precipitation of BAC DNA (for up to 100 mL culture) or **Section 3** for column purification of BAC DNA (for up to 5 mL culture)

Section 2. Alcohol Precipitation of BAC DNA for Cultures Up to 100 mL

2. Alcohol Precipitation of BAC DNA

a. Add an equal amount of cold **Isopropanol** according to **Table 2** to the lysate prepared from Step 1f and mix well by inverting 10 times.

Table 2. Amount of Solution Required for Alcohol Precipitation

Input Culture	1.5 mL	5 mL	10 mL	40 mL	100 mL
Isopropanol	900 µL	2.25 mL	4.5 mL	18 mL	45 mL

b. Incubate on ice or at -20°C for 5 minutes.

c. Centrifuge for 10 minutes at 14,000 x g (14000 RPM) at 4°C.

d. Pour off supernatant. **For Input Culture Volumes up to 5 mL**, resuspend pellets in 0.5 mL of **TE Buffer**. **For Input Culture Volumes larger than 5 mL**, resuspend pellets in 2 mL of **TE Buffer**. Mix by gentle tapping or swirling of the tube or bottle.

e. Add 2 µL of **RNase**. Mix by gentle tapping or swirling. Incubate at 37°C for 10 minutes.

f. **For Input Culture Volumes up to 5 mL**, add 1 mL of 96 – 100% Ethanol. **For Input Culture Volumes larger than 5 mL**, add 4 mL of 96 – 100% Ethanol. Mix well by inverting 10 times.

g. Centrifuge for 5 minutes at 14,000 x g (14000 rpm) at 4°C.

h. Pour off supernatant. Air dry the pellet at room temperature for 3 – 5 minutes.

i. Resuspend the BAC DNA in an appropriate amount of TE as suggested by **Table 3**.

Table 3. Recommended Volume of TE for Resuspending Final BAC DNA Pellet

Input Culture	1.5 mL	5 mL	10 mL	40 mL	100 mL
TE Buffer	25 µL	50 µL	50 µL	200 µL	200 µL

Section 3. Column Purification of BAC DNA for Cultures Up to 5 mL

Notes prior to use:

- This protocol is only applicable for input culture volume of up to 5 mL.
- The protocol is best suited for downstream applications that are sensitive to contaminants from alcohol precipitation such as sequencing and PCR
- Due to the nature of column purification, the purified DNA may contain only very small percentage of intact super-coiled BAC DNA

2. Binding to Column

a. Add an appropriate amount of **RNase** according to **Table 4** to the lysate. Mix by gentle tapping or swirling. Incubate at 37°C for 10 minutes.

b. Add an appropriate amount of **Binding Solution** according to **Table 4** to the lysate prepared from Step **1f** and mix well by vortexing.

Table 4. Amount of Solutions Required for Column Purification

Input Culture	1.5 mL	5 mL
RNase	2 µL	7.5 µL
Binding Solution	100 µL	250 µL
96 – 100% Ethanol	100 µL	250 µL

c. Add an appropriate amount of 96 – 100% Ethanol according to **Table 4**. Mix well by vortexing

d. Retrieve a spin column assembled with a collection tube.

e. Transfer the lysate into the spin column, cap the column and then centrifuge the unit for 1 minute at 6,700 x g (8,000 RPM).

f. After centrifugation, separate the column from its collection tube. Discard the flowthrough and reassemble the spin column with its collection tube.

g. Repeat Steps **2d** and **2e** until all lysate has passed through the column.

3. Washing Bound DNA

a. Apply 500 µL of **Wash Solution** to the column, and centrifuge for 1 minutes at 14,000 x g (14,000 RPM).

b. Discard the flowthrough and reassemble the column with the collection tube.

c. Repeat Steps **3a** and **3b** one more time.

d. Spin the column for 2 minutes at 14,000 x g (14,000 RPM) in order to thoroughly dry the column. Discard the collection tube.

4. Elution of Clean DNA

a. Assemble the spin column with a provided **Elution Tube.**

b. Add 100 µL of **Elution Buffer** to the center of the column. Wait for 1 minute. Centrifuge the assembly for 1 minute at **14,000 *x g* (14,000 RPM).** The elution contains the pure BAC DNA

c. (Optional): An additional elution can be performed by repeating steps **4a** and **4b**. 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.
	Proper amount of Isopropanol or Ethanol was not used in alcohol precipitation	Ensure that the appropriate amount of alcohol was used in each step indicated. The ratio of alcohol recommended is optimized for high recovery
DNA appeared to be sheared	Handling of lysate was not gentle	Due to the size of BAC, the DNA could be easily sheared by vortex or repeated pipetting after cell lysis. Mix the lysate according to what is suggested by the protocol. Avoid vortex or repeated pipetting if necessary
	Column purification may result in lower % of intact super-coiled BAC	Due to the nature of the column-based procedure, the purified DNA may contain only a very small percentage of intact super-coiled BAC DNA. If intact BAC is desired, only the Alcohol Precipitation protocol should be used
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

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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