

## BAC DNA MiniPrep Kit

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 mg 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.



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. **For all input culture volumes (up to 100 mL) and downstream applications that require intact plasmid**, the alcohol precipitation protocol is to be followed. In this procedure, a series of alcohol precipitations and washes are performed to obtain clean, intact plasmid DNA. **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. The column purification method relies on the use of spin column chromatography. 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.

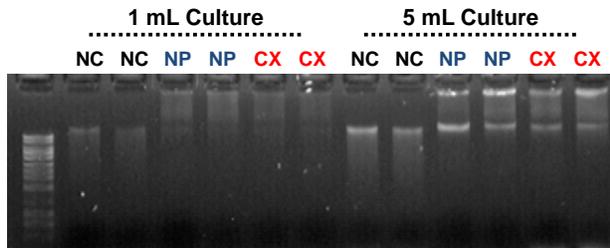
This kit is designed to process either 50 x 1.5 mL culture samples, 30 x 5 mL culture samples, 15 x 10 mL culture samples, 6 x 40 mL culture samples or 3 x 100 mL culture samples.

| Kit Specifications                             |               |  |            |
|--|---------------|--|------------|
| Input Culture Volume for Alcohol Precipitation | Up to 100 mL  | Input Culture Volume for Column Purification | Up to 5 mL |
| Column Binding Capacity                        | 25 µg         | Average Yield from 3 mL of Culture           | 0.6 - 1 µg |
| Size of Plasmids Purified                      | Up to 130 kbp | Time to Complete 10 Purifications            | 1 hour     |

### BAC DNA MiniPrep Kit Benefits

|   |  |
|---|--|
| Fast and easy processing                              | Rapid alcohol precipitation or spin-column purification allows for the processing of multiple samples in 1 hour. |
| High binding capacity of columns                      | The binding capacity of the columns in the BAC DNA MiniPrep Kit is 25 µg.  |
| High recovery   | 0.6 to 1 µg of BAC DNA can be purified from 3.0 mL of bacterial culture.   |
| Recovered DNA is suitable for downstream applications | Purified BAC DNA is fully compatible with restriction enzyme digestions and manual or automated sequencing.      |

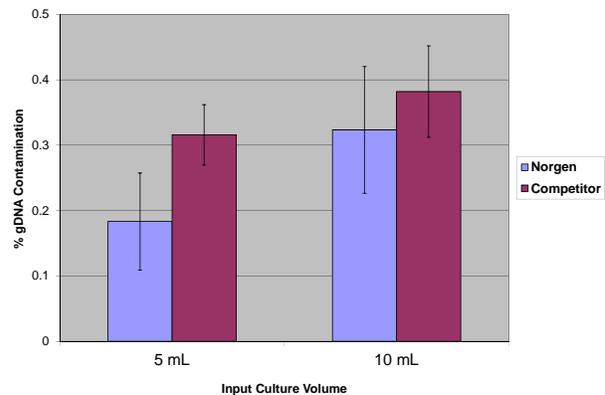
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**Figure 1. High Yield of BAC DNA by Alcohol Precipitation or Column Purification.** BAC DNA was isolated from cultures of 1 and 5 mL by both alcohol precipitation and column purification using Norgen's BAC DNA Isolation Kit and compared to a competitor's kit that uses alcohol precipitation. Ten micro-liter of 100  $\mu$ L DNA was loaded on a 1X TAE 0.6% Agarose DNA gel with 2.5 mL of Norgen High-Ranger as MW Marker. DNA isolated by Norgen's alcohol precipitation protocol (NP) yielded better amounts of high MW intact BAC DNA, compared to that isolated with competitor's product (CX). Norgen's alternative column-based procedure (NC) also isolated high yield of DNA. NC = Norgen's column purified DNA, NP = Norgen's alcohol precipitation DNA and CX = competitor purified DNA.

### BAC DNA MiniPrep Kit Contents

1. Resuspension Buffer
2. Lysis Solution
3. Neutralization Solution
4. Binding Solution
5. RNase
6. TE Buffer
7. Wash Solution
8. Elution Buffer
9. Spin Columns inserted in Collection Tubes
10. Elution Tubes
11. User Manual



**Figure 2. High Quality of BAC DNA with Minimum Amount of Genomic DNA Contamination.** Norgen's BAC DNA Isolation Kit isolates high quality of BAC DNA with minimum amount of genomic DNA contamination. BAC DNA was isolated from 5 and 10 mL cultures using Norgen's BAC DNA Isolation Kit and compared to a competitor's kit that uses alcohol precipitation. Total DNA yield was quantified by GE's NanoVue. Genomic DNA of *E. coli* was quantified by quantitative PCR. The percentage of *E. coli* genomic DNA contamination was calculated as the percentage of quantified *E. coli* genomic DNA over total DNA, and was found to be much lower for Norgen purified BAC DNA compared to competitor purified BAC DNA.

### Storage Conditions

All solutions should be kept tightly sealed and stored at room temperature. The RNase A and should be stored at -20°C upon arrival. All the reagents should remain stable for at least 1 year in their unopened containers.

### Shipping Conditions

The BAC DNA MiniPrep Kit is shipped at room temperature.

| Cat # | Description          | Quantity          |
|-------|----------------------|-------------------|
| 18050 | BAC DNA MiniPrep Kit | 50 preps x 1.5 mL |