

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. 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 Solution A. Lysis Buffer N is then added to the sample in order to assist in the lysis of the bacterial cells. Next, Neutralization Solution A is added to the sample which will neutralize the sample and cause precipitation of the proteins and genomic DNA that is present

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.

BAC DNA MiniPrep Kit

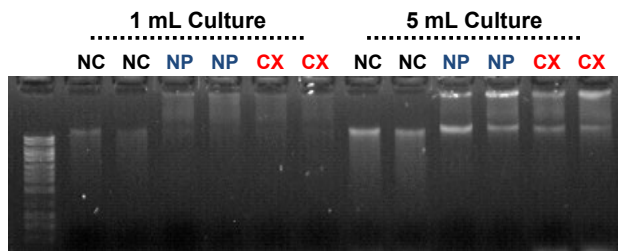


Figure 1. High Yield of BAC DNA by Alcohol Precipitation or Column Purification. BAC DNA was isolated from cultures of 1 and 5 mL by both alcohol precipitation and column purification using Norgen's BAC DNA Isolation Kit and compared to a competitor's kit that uses alcohol precipitation. Ten micro-liter of 100 μ 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 Solution A
2. Lysis Buffer N
3. Neutralization Solution A
4. Solution BX
5. Wash Solution A
6. Elution Buffer M
7. TE Buffer
8. RNase
9. Spin Columns inserted in Collection Tubes
10. Elution tubes (1.7 mL)
11. Product Insert

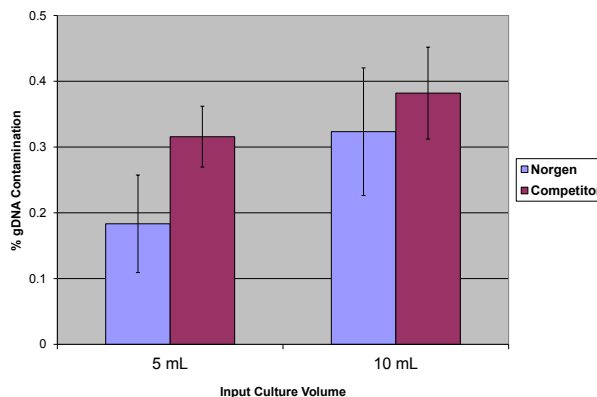


Figure 2. High Quality of BAC DNA with Minimum Amount of Genomic DNA Contamination. Norgen's BAC DNA Isolation Kit isolates high quality of BAC DNA with minimum amount of genomic DNA contamination. BAC DNA was isolated from 5 and 10 mL cultures using Norgen's BAC DNA Isolation Kit and compared to a competitor's 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