



User Bulletin

Mini Plus™ BAC DNA Extraction System

Viogene Mini Plus™ BAC DNA Extraction System provides a simple, fast and cost-effective method to purify BAC DNA without phenol/chloroform extraction. It is based on bind-wash-elute of BAC DNA from silica-based membrane columns. An average yield of 0.5 to 3 µg of BAC DNA can be expected from 1 to 5 ml overnight bacterial culture.

Downstream Application

- * Restriction digestion
- * Radioactive and fluorescent sequencing
- * Transformation
- * Ligation
- * PCR, RAPD

Product Contents

Cat. No	BAC1001	BAC1002
Preps	50	250
BX1 Buffer	12	60
BX2 Buffer	15	75
BX3 Buffer	20	100
WN Buffer	6	30
WS Buffer	10	45
Elution Buffer	5	25
RNase A (20mg/ml)	0.042ml	0.21ml
Mini Plus™ Column	50	250
Collection Tube	50	250
Protocol	1	1

All buffers need to be mixed well before use.

Shipping & Storage

The sample of Mini Plus™ BAC DNA Extraction System is shipped and should be stored at ambient temperature up to 6 months.

If precipitate form by freezing temperature on any buffer, warm up at 37°C to redissolve.

Protocol

❖ **Please read the following notes before starting the procedures.**

Important Notes

- Add all of RNase A (20mg/ml, 0.042 or 0.210ml) into the BX1 Buffer and mix well, store at 4°C

For BAC1001

- Add 24 ml of 98 - 100 % ethanol into WN Buffer bottle / 40 ml of 98 - 100 % ethanol into WS Buffer bottle when first open.

For BAC1002

- Add 150 ml of 98 - 100 % ethanol into WN Buffer bottle / 180 ml of 98 - 100 % ethanol into WS Buffer bottle when first open.
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- If precipitation forms in BX2 Buffer, incubate the buffer at 55 °C for 10 minutes to redissolve the salt precipitates.
- Do not shake BX2 Buffer, SDS in BX2 will lead to serious foaming.
- All procedures should be done at room temperature (20 - 25 °C).
- For long-term storage of the eluted BAC, TE buffer should be used for elution. Since EDTA in TE may affect downstream applications, Elution Buffer (provided) or ddH₂O (pH 7.0 - 8.5) is preferred for DNA elution immediately used for further enzymatic reactions.

- **Viogene's unique design – EasyLid™**

The EasyLid™ is designed to prevent contamination during the procedure.

Tips for EasyLid™ –

Twist the arm of the cap and pull the cap to break the EasyLid™.



I. Protocol for Spin Method:

1. **Grow 1 to 5 ml BAC DNA-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.**
2. **Pellet the cells by centrifuging for at 10,000 x g (13,000 rpm) 1 - 2 minutes. Decant the supernatant and remove all medium residue by pipetting.**
3. **Add 200 µl of BX1 Buffer to the pellet, resuspend the cells completely by vortexing or pipetting.**
No cell clumps should be visible after resuspension of the pellet.
4. **Add 250 µl of BX2 Buffer and gently mix (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1 - 5 minutes.**
Do not vortex, vortexing will shear genomic DNA.
5. **Add 350 µl of BX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.** The white precipitate should be formed.
6. **Centrifuge at 10,000 x g (13,000 rpm) for 5 - 10 minutes, place a Mini Plus™ Column onto a Collection Tube.**
7. **Meanwhile, heat up desired volume of Elution Buffer (provided) at 70°C bath.**
8. **Transfer the supernatant of step 6 carefully into the Mini Plus™ column after centrifugation.**
9. **Centrifuge at 3,000 x g (4,000 rpm) for 1-2 minutes. Discard the flow-through. It is important using low speed centrifugation to avoid BAC DNA shearing.**
10. **Wash the column once with 0.5 ml WN Buffer by centrifuging at 3,000 x g (4,000 rpm) for 1-2 minutes. Discard the flow-through.**
11. **Wash the column once with 0.7 ml WS Buffer by centrifuging at 3,000 x g (4,000 rpm) for 1-2 minutes. Discard the flow-through.**
12. **Centrifuge the column at 3,000 x g (4,000 rpm) for another 5 minutes to remove residual ethanol.**
It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

13. Place the column onto a new 1.5-ml centrifuge tube. Add 30 μ l of 70°C heated Elution Buffer onto the center of the membrane.

For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

14. Stand the column for 1 minutes and centrifuge at 3,000 x g (4,000 rpm) for 2 - 3 minutes to first elute BAC DNA.

15. Add another 20 μ l of 70°C heated Elution Buffer onto the center of the membrane and centrifuge 2 - 3 minutes at 3,000 x g (4,000 rpm) for secondary BAC DNA elution.

16. Store BAC DNA at 4 °C or -20 °C.

II. Protocol for Vacuum Method:

1. Grow 1 to 5 ml BAC DNA-containing bacterial cells in LB medium with appropriate antibiotic(s) overnight (12-16 hours) with vigorous agitation.

2. Pellet the cells by centrifuging for 1 - 2 minutes. Decant the supernatant and remove all medium residue by pipetting.

3. Add 200 μ l of BX1 Buffer to the pellet, resuspend the cells completely by vortexing or pipetting.

No cell clumps should be visible after resuspension of the pellet.

4. Add 250 μ l of BX2 Buffer and gently mix (invert the tube 4-6 times) to lyse the cells until the lysate becomes clear. Incubate at room temperature for 1 - 5 minutes.

Do not vortex, vortexing will shear genomic DNA.

5. Add 350 μ l of BX3 Buffer to neutralize the lysate, then immediately and gently mix the solution.

The white precipitate should be formed.

6. Centrifuge at 10,000 x g (13,000 rpm) for 5 - 10 minutes, meanwhile insert the tip of a Mini *Plus*TM Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*).

Meanwhile, heat up desired volume of Elution Buffer (provided) at 70°C bath.

7. Transfer the supernatant carefully into a Mini *Plus*TM column.

8. Apply vacuum to draw all the liquid into the manifold.

9. Wash the column once with 0.5 ml WN Buffer by re-applying vacuum to draw all the liquid.

10. Wash the column once with 0.7 ml WS Buffer by re-applying vacuum to draw all the liquid.

It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

11. Centrifuge the column at 3,000 x g (4,000 rpm) for 5 minutes to remove residual ethanol.

It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

12. Place the column onto a new 1.5-ml centrifuge tube. Add 30 μ l of 70°C heated Elution Buffer onto the center of the membrane.

For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed.

13. Stand the column for 1 minutes and centrifuge at 3,000 x g (4,000 rpm) for 2 - 3 minutes to first elute BAC DNA.

14. Add another 20 μ l of 70°C heated Elution Buffer onto the center of the membrane and centrifuge 2 - 3 minutes at 3,000 x g (4,000 rpm) for secondary BAC DNA elution.

15. Store BAC DNA at 4 °C or -20 °C.

* Vac-man is a trademark of Promega Corporation.