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VIOGENE

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℃ 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℃

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 ℃ 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 ℃).
- 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 ddH_2O (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℃ 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 μl of 70℃ 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 1minutes and centrifuge <u>at 3,000 x g (4,000 rpm)</u> for 2 - 3 minutes to first elute BAC DNA.**

15. **Add another 20 μl of 70℃ heated Elution Buffer onto the center of the membrane and centrifuge 2 - 3 minutes <u>at 3,000 x g (4,000 rpm)</u> for secondary BAC DNA elution.**

16. **Store BAC DNA at 4 ℃or –20 ℃.**

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 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 <u>at 10,000 x g (13,000 rpm)</u> for 5 - 10 minutes, meanwhile insert the tip of a Mini *Plus*™ 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℃ bath.

7. **Transfer the supernatant carefully into a Mini *Plus*™ 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 <u>at 3,000 x g (4,000 rpm)</u>** 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 μl of 70℃ 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 1minutes and centrifuge at 3,000 x g (4,000 rpm) for 2 - 3 minutes to first elute BAC DNA.**

14. **Add another 20 μl of 70℃ 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 ℃or –20 ℃.**

* Vac-man is a trademark of Promega Corporation.