PowerPrep™ HP BAC Buffer Kit

Store at RT

Catalog No. NP100003N (11448-100)

NOTICE: Kit NP100003N does not include any buffers listed. A protocol detailing how to create buffers necessary is provided.

Overview

Preparation of BAC (Bacterial Artificial Chromosome) DNA with OriGene's PowerPrep™ HP Plasmid Purification kits requires additional volume of several of the buffers included in the purification kits. The HP BAC Buffer Kit supplies the necessary additional buffer volumes for preparation of high purity BAC DNA. Cell Suspension Buffer, Cell Lysis Solution, Neutralization Buffer and RNase A are included in the HP BAC Buffer Kit.

We have achieved yields of a ~100 kb BAC molecule of approximately 40 µg DNA/100 mL culture with this procedure.

Materials Required but Not Supplied

This protocol requires OriGene's PowerPrep™ HP plasmid purification systems. Any of the following kits can be used:

ITEM NAME	CAT. NO.	SIZE
PowerPrep™ HP Plasmid Miniprep System	NP100004	25 Reactions
	NP100005	100 Reactions
PowerPrep™ HP Plasmid Midiprep System	NP100006	25 Reactions
	NP100007	50 Reactions
PowerPrep™ HP Plasmid Maxiprep System	NP100008	10 Reactions
	NP100009	25 Reactions

Components

COMPONENT NAME	VOLUME
Cell Suspension Buffer	250 mL
Cell Lysis Solution	250 mL
Neutralization Buffer	200 mL
RNase A	2 x 28mL

Safety and Use Statement

This product contains hazardous reagents. It is the end-user's responsibility to consult the applicable MSDS(s) before using this product. Disposal of waste organics, acids, bases, and radioactive materials must comply with all appropriate federal, state, and local regulations. If you have questions concerning the hazards associated with this product, please call OriGene at (888) 267-4436.

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

Before Beginning

- Before beginning prepare a 20 h culture of BAC containing bacteria in 2X YT and appropriate antibiotic. The OD_{600} of the final culture should be 5.0 ± 0.5.
- Add 5.0 mL of **RNase A** to **Cell Suspension Buffer** to a final concentration of **400 µg/mL**. NOTE: In this application, we recommend 400 µg RNase A/mL of Cell Suspension Buffer because of the extremely low copy number of BACs.
- Increase NaCl concentration in **Wash Buffer** from 0.825 M to 0.9 M NaCl by adding 0.58 g NaCl per 100 mL Wash Buffer. Conductivity should be 72 mS. This increase in salt will reduce the RNA contamination in the BAC prep.
- Pre-warm **Elution Buffer** to 50°C.

Use the volumes recommended in this procedure for optimal performance.

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Protocol

1. Equilibrate the column with **Equilibration Buffer**. Allow the solution in the column to drain by gravity flow.

Volume of Equilibration Buffer	Mini	Midi	Maxi
	2 mL	10 mL	30 mL

2. Pellet the cultured cells by centrifugation at 9,000 x *g* for 15 min. THOROUGHLY remove all media after pelleting and before resuspending.

Volume of BAC culture	Mini	Midi	Maxi
	10-25 mL	100 mL	200-500 mL

3. Resuspend the cells in **Cell Suspension Buffer** containing 400 µg/mL RNase A.

Volume of Cell Suspension Buffer	Mini	Midi	MAXI
	2 mL	8 mL	40 mL

4. Lyse the cells with **Cell Lysis Solution**. Mix IMMEDIATELY. Mix gently but thoroughly until a homogenous mixture is obtained. Due to the release of genomic DNA, the mixture is very viscous at this stage. Incubate at room temperature for 5 min.

Volume of Cell Lysis Solution	Mini	Midi	Maxi
	2 mL	8 mL	40 mL

5. Neutralize the lysis mixture by adding **Neutralization Buffer**. Mix IMMEDIATELY. Mix thoroughly, but do not vortex. Vortexing can result in shearing of the DNA.

Volume of Neutralization Buffer	Mini	Midi	Maxi
	2 mL	8 mL	40 mL

6. Centrifuge the lysate for 10 min >15,000 x *g* at room temperature. Collect the supernatant into a fresh tube. Collection of the supernatant should be performed with a pipet, not by decanting.

7. Apply the cleared lysate supernatant to the column. Allow the lysate to run through the column by gravity flow. DO NOT force out the remaining buffer.

8. Wash the column once with **Wash Buffer**. Allow the wash to flow through the column by gravity. Discard the flow-through.

Volume of Wash Buffer	Mini	Midi	Maxi
	2.5 mL	10 mL	60 mL

9. Elute the DNA from the column into a clean tube by adding **Elution Buffer** that has been warmed to 50°C. Allow the solution to drain by gravity flow. *Do not force out remaining solution. Prewarming of Elution Buffer will enhance the release of high-molecular weight DNA.*

Volume of Elution Buffer	Mini	Midi	Maxi
	0.9 mL	5 mL	15 mL

10. Precipitate the DNA by adding isopropanol to the eluate. Mix and centrifuge DNA for 30 min at >12,000 x *g* at 4°C. Carefully discard the supernatant.

Volume of iso-propanol	Mini	Midi	Maxi
	0.63 mL	3.5 mL	10.5 mL

11. Wash the BAC DNA pellet with 80% ethanol and centrifuge at >15,000 x *g* at 4°C for 5 min. Carefully and fully pipet off the ethanol wash. Air dry the pellet for 10 min. Dissolve the DNA in **TE Buffer**.

Volume of TE Buffer	Mini	Midi	Maxi
	10 µL	50-100 µL	200-400 µL

Technical Support

For further technical assistance please contact us at (888) 267-4436 or by email at techsupport@origene.com. Technical support and troubleshooting guides for these products can also be found on our website at www.origene.com.

Related Products

To see our full line of PowerPrep™ purification products visit our website at www.origene.com.