BIOMIGA®
倍沃医学科技

Ver: 1908

BW-PD1311 EZgene™ BAC/PAC Miniprep Kit

Contents

Kit Contents

Catalog#	BW-PD1311-00	BW-PD1311-01	BW-PD1311-02
Preps	10	50	250
Mini Columns	10	50	250
2 mL Collection Tubes	10	50	250
Buffer X1	5 mL	25 mL	125 mL
Buffer X2	5 mL	25 mL	125 mL
Buffer X3	5 mL	25 mL	125 mL
BAC Binding Buffer*	1 mL	5 mL	25 mL
Elution Buffer	1 mL	5 mL	25 mL
DNA Wash Buffer**	3 mL	12 mL	50 mL
RNase A (20 mg/mL)	45 µL	225 µL	1125 µL
User Manual	1	1	1

*Add 4 mL (BW-PD1311-00) or 20 mL (BW-PD1311-01) or 100 mL (BW-PD1311-02) 96-100% isopropanol to each BAC Binding Buffer bottle before use.
**Add 12 mL (BW-PD1311-00) or 48 mL (BW-PD1311-01) or 200 mL (BW-PD1311-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

The EZgene™ BAC/PAC Miniprep Kit is designed for rapid purification of cosmid, BAC, PAC and P1 from small volume of bacterial cultures. It is based on a modified alkaline lysis procedure that is specially adapted for spin column. The procedure associated with this kit has been tested using a variety of low copy cosmid, BAC, PAC and P1 in different *E. coli* strains. In addition, this kit can also be used for high copy plasmid isolation.

Storage and Stability

Buffer X1 should be stored at 4℃ once RNase A is added. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

Important Notes

✪ RNase A: 20 mg/mL. It is stable for one year at room temperature (15-25℃). Spin down RNase A vial briefly before adding to Buffer X1.

✪ Buffer X1 should be stored at 4℃ once RNase A is added.

✪ Add 12 mL (BW-PD1311-00) or 48 mL (BW-PD1311-01) or 200 mL (BW-PD1311-02) 96-100% ethanol to DNA Wash Buffer bottle before use.

✪ Add 4 mL (BW-PD1311-00) or 20 mL (BW-PD1311-01) or 100 mL (BW-PD1311-02) 96%-100% isopropanol to Dilute BAC Binding Buffer bottle before use.

✪ It's strongly recommended to use 2× YT media for the cultivation of cosmids, BACs, PACs, and P1s.

✪ Buffer X2 should be kept at room temperature. Check for SDS precipitation before use. If necessary re-dissolve SDS precipitate by warming. Keep the cap tightly closed for Buffer X2 after use to avoid acidification that may result from air CO_2.

✪ Chill Buffer X3 for precipitation enhancement.

✪ Prewarm ddH_2O or Elution Buffer at 65℃ before elution.

✪ Use 4℃ microcentrifuge for step **6**.

Materials not Supplied

✪ Microcentrifuge capable of at least 12,000 ×g.

✪ Microcentrifuge set at 4℃ capable of at least 12,000 ×g.

✪ Sterile deionized water.

✪ Sterile 1.5 mL and 2 mL centrifuge tubes.

✪ 10-15 mL culture tubes.

✪ 96-100% ethanol.

✪ 96-100% isopropanol.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

BAC, PAC, and P1 purification protocol

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a starter culture of 2-5 mL LB or 2 mL TB medium containing the appropriate selective antibiotic. Incubate for 20-24 h at 37℃ with vigorous shaking (~ 300 rpm). Use a flask with a volume at least 4 times the volume of the culture.
2. Pellet ***2-5 mL*** bacteria by centrifugation at 12,000 ×g for 2 minutes at room temperature. Decant or aspirate medium and discard.
3. Resuspend the bacterial pellet by adding **400 µL Buffer X1/RNase A** solution, and vortexing. Complete resuspension of cell pellet is vital for obtaining good yields. Transfer the resuspended bacterial into a 2 mL tube.
4. Add **400 µL Buffer X2** and mix gently but thoroughly by inverting 5-10 times to obtain a clear lysate. Incubate at room temperature for 5 minutes. Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. The lysate should appear viscous. Do not incubate more than 5 minutes. (Store **Buffer X2** tightly capped).
5. Add **400 µL Buffer X3** (chilled) and gently but thoroughly mix the sample by inverting 10-15 times until a flocculent white precipitate forms. Incubate on ice for 5 minutes.
6. Centrifuge at 12,000 ×g for 10 minutes at 4℃. Promptly proceed to the next step.
7. Carefully transfer the clear supernatant to a new 2 mL tube. Add **450 µL BAC Binding Buffer**.

   Note: Add isopropanol to BAC Binding Buffer before use.
8. Transfer **700 µL** of the sample to the **Mini Column**. Centrifuge at 12,000 ×g for 15 seconds at room temperature. Discard the flow-through. Transfer the remaining sample to the column and centrifuge at 12,000 ×g for 30 seconds at room temperature. Discard the flow-through.
9. Add **700 µL DNA Wash Buffer**. Centrifuge at 12,000 ×g for 30 seconds at room temperature. Discard the flow-through.
10. Place the **Mini Column**, **with the lid open**, back into the collection tube and centrifuge at 12,000 ×g for 1 minute to remove residual ethanol.
11. Place the **Mini Column** into a clean 1.5 mL centrifuge tube, add **35-50 µL** pre-warmed (65℃) **Elution Buffer** or ddH_2O onto the center of the membrane. Incubate 5 minutes.

12. Centrifuge at 12,000 ×g for 1 minute to elute the DNA. Add the eluted DNA back to the column and centrifuge at 12,000 ×g for 1 minute to elute the DNA.

    Note: Pre-warm Elution Buffer or ddH_2O at 65°C and incubate the column at 65°C for 5 minutes after adding Elution Buffer or ddH_2O will increase the DNA yield.

    Note: The first elution normally yields 60-70% of the DNA. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA.

13. Store the eluted DNA at -20°C.

Purification of Low-Copy-Number Plasmid/Cosmid

Expected yield: The yield of BAC is around 0.6 µg from 2 mL LB culture and 1 µg from 5 mL culture. If cultured in TB, the yield is about 1 µg from 1.5 mL culture and 3 µg from 5 mL culture.

Culture volume: Use a flask or tube with a volume at 4 times the culture medium to secure optimal condition for bacteria growth. Don't exceed the maximum culture volume suggested in the protocol. Incomplete lysis due to over amount of bacterial culture results in lower yield and less purity.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low DNA yield	Poor cell lysis	Only use LB or TB medium containing ampicillin. Do not use more than 5 mL culture with the basic protocol.
		Cells may not have been dispersed adequately prior to the addition of Buffer X2. Make sure to vortex cell suspension to completely disperse.
		Continue inverting vials after adding Buffer X2 to obtain a clear lysate.
		If not tightly closed, Buffer X2 may need to be replaced. Prepare as follows: 0.2 M NaOH, 1% SDS.
	Bacterial clone is not fresh	Use fresh glycerol cultures and avoid repeated freezing/thawing cycles of clones. Always make enough replica plates and use fresh cultures for inoculation. Any remaining cultures can be used to set up fresh glycerol stocks.
No DNA	Lysate prepared incorrectly	Check the stock of buffers and age of the buffers. Make sure that the correct volume of buffer has been added to the samples.
	Buffer X2 precipitated	Warm up the Buffer X2 to dissolve the precipitate.
	Cells are not completely resuspended	Pelleted cells should be completely resuspended with Buffer X1. Do not add Buffer X2 until an even cell suspension is obtained.
High molecular	Over mixing of cell lysate	Do not vortex or mix aggressively

weight DNA contamination of product.	upon addition of X2	after adding Buffer X2.
	Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours.
DNA degraded after the storage	High levels of endonuclease activity.	Perform the heat inactivation step.
RNA visible on agarose gel	RNase A not added to Buffer X1.	Add 1 vial of RNase A to each bottle of Buffer X1.
	DNA floats out of well while loading agarose gel.	Air dry the DNA pellet before re-dissolving the DNA.

Limited Use and Warranty

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at 400-115-2855 or visit our website at www.biomiga.com.cn