Ver: 2003

BW-MPD1220 EndoFree Plasmid Miniprep Kit(Magnetic beads)

Contents

Kit Contents

Catalog#	BW-MPD1220-A00-00	BW-MPD1220-A00-01	BW-MPD1220-A00-02
Preps	50	250	1000
Plasmid-L Beads	2 x 900 µL	8.8 mL	35.5 mL
Buffer A1	15 mL	65 mL	255 mL
Buffer B1	15 mL	65 mL	255 mL
Buffer N3	5 mL	20 mL	80 mL
Buffer KB	30 mL	130 mL	500 mL
Buffer RET	15 mL	65 mL	255 mL
DNA Wash Buffer*	15 mL	2 x 40 mL	3 x 100 mL
RNase A (20 mg/mL)	120 µL	520 µL	2 x1.1 mL
EndoFree Elution Buffer	10 mL	50 mL	200 mL
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*Add 60 mL(BW-MPD1220-A00-00) or 160 mL(BW-MPD1220-A00-01) or 400 mL(BW-MPD1220-A00-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

Key to the plasmid purification kit is our proprietary DNA binding system that allows the high efficient binding of DNA to our Magnetic beads while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with sterile water or EndoFree Elution Buffer. The purified DNA is guanidine/anion exchange resin residues free.

The EndoFree system uses a specially formulated buffer that extracts the endotoxin from the bacterial lysate. The endotoxin level is 1 to 10 EU (Endotoxin) per µg.

This kit is designed for fast and efficient purification of plasmid DNA from 1 to 5 mL of *E. coli* culture. The Magnetic beads has a DNA binding capacity of 50 µg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

The kit can be matched with a variety of automated nucleic acid extractors, such as Allsheng Auto-Pure 96A.

Important Information

Plasmid Copy Numbers: The yield of plasmid DNA depends on the origin of the replication and

the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up accordingly. Please contact our customer service for further information and refer to Table 1 for the commonly used plasmids.

Table 1 Commonly used plasmids and expected yield.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 1 mL)
pSC101	pSC101	5	0.1-0.2
pACYC	p15A	10-12	0.4-0.6
pSuperCos	pMB1	10-20	0.4-1
pBR322	pMB1	15-20	0.6-1
pGEM^{R}	Muted pMB1	300-400	6-7
pBluescript^{R}	ColE1	300-500	6-8
pUC	Muted pMB1	500-700	8-12

Host Strains: The strains used for propagating plasmid have significant influence on yield. The strains used for propagating plasmid have significant influence on yield. Host strains such as TOP10, DH5α and C600 yield high-quality plasmid DNA. *EndA*+ strains such as JM101, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommended transform plasmid to an *endA*- strain if the yield is not satisfactory. Please refer to Table 2 for the *endA* information.

Table 2 *endA* strains of *E. coli*.

End A- Strains of *E.coli*							
DH5α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2^{TM}	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96^{TM}	Stbl4^{TM}	XL10-Gold
End A+ Strains of *E. coli*							
C600	JM110	RR1	ABLE$^{®}$C	CJ236	KW251	P2392	BL21(DE3)
HB101	TG1	TB1	ABLE$^{®}$K	DH12S^{TM}	LE392	PR700	BL21(DE3) pLysS
JM101	JM83	TKB1	HMS174	ES1301	M1061	Q358	BMH71-18
All NM Strains				All Y Strains			

Optimal Cell Mass (OD_{600} × mL of Culture): This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) for 12-16 hours to a density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2 × YT are used, make sure the cell density doesn't exceed 3.0 (OD_{600}). A high ratio of biomass over lysis buffers result in low DNA yield and purity. The Mini Column

has an optimal biomass of 10-15. For example, if the OD_{600} is 2.5, the optimal culture volume should be 1-5 mL. For over amount of cell numbers, either reduce the biomass or scale up the volume of Buffer A1, B1, N3 and RET.

Culture Volume: Use a flask or tube 4 times bigger in volume than 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 purity.

Storage and Stability

Buffer A1 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 A1.

✪ Buffer A1 should be stored at 4℃ once RNase A is added.

✪ Add 60 mL(BW-MPD1220-A00-00) or 160 mL(BW-MPD1220-A00-01) or 400 mL(BW-MPD1220-A00-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

✪ Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 37℃ to dissolve the precipitates before use. Keep the cap tightly closed for Buffer B1 after use.

✪ Plasmid-L beads: Vortex well before use. It is recommended to divide according to its own use, avoid repeated opening and vortexing to reduce the magnetic bead magnetism, and increase the magnetic bead fragments

✪ Buffer N3 may form precipitates upon storage, warm up at 37℃ to dissolve the precipitates before use.

✪ Ensure the availability of centrifuge capable of 12,000 ×g.

✪ *Carry out all centrifugations at room temperature.*

Materials not Supplied

✪ High speed centrifuge.

✪ 96-100% ethanol.

✪ 1.5 mL centrifugal tubes.

Safety Information

Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling.

Buffer RET contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

EndoFree Plasmid Miniprep （Magnetic beads）Protocol

1. Inoculate ***1-5 mL*** LB containing appropriate antibiotic with 100 µL fresh starter culture. Incubate at 37℃ for 14-16 hours with vigorous shaking.

   Note: Prolonged incubation (>16 hours) is not recommended since the *E. coli* starts to lyse and the plasmid yield may be reduced.

   Note: Do not grow the culture directly from the glycerol stock.

   Note: Do not use a starter culture that has been stored at 4℃.

   Note: This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2 × YT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers need to be scaled up proportionally if over amount of cultures are being processed.

2. Harvest the bacterial culture by centrifugation for 1 minutes at 12000 ×g. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   Note: Residue medium will cause poor cell lysis and thus lower DNA yield.

3. Add **250 µL Buffer A1** (*Add **RNase A** to Buffer A1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting.

   Note: Complete resuspension is critical for bacterial lysis and lysate neutralization.

4. Add **250 µL Buffer B1**, mix gently by inverting the tube 10 times (*do not vortex*), and incubate at room temperature for 5 minutes.

   Note: Do not incubate for more than 5 minutes.

   Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 37℃ to dissolve the precipitations before use.

5. Add **75 µL Buffer N3**, mix completely by inverting/shaking the vial for 5-10 times.

   Note: Incubating the lysate in ice for 1 minute will improve the yield.

   Note: It is critical to mix the solution well. If the mixture still appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution.

6. Transfer the lysate to a high speed centrifuge tube and centrifuge at 12,000 ×g for 10 minutes at room temperature. Transfer the cleared lysate to a 1.5 mL centrifugal tube (avoid the floating precipitates).

   Note: If the rotor is cold, incubate the lysate at room temperature for 10 minutes and then perform centrifugation as described.

7. Add **250 µL Buffer RET** and **250 µL** 100% isopropanol. Mix immediately by sharp shaking.

8. Immediately add **35ul plasmid-L beads** to the step7 **Collection Tube**. Shaking for 5min at RT，then place the tube on an Magnet and allow beads to separate. With the tube on the Magnet, perform the aspiration, and then discard the supernatant from the tube.

9. **Optional:** Add **500 µL Buffer KB** into the **tube**, shaking for 1 minute, discard the supernatant. Put the tbue back to the Magnet.

   Note: This step is NOT necessary if the plasmid is being purified from *endA*- strain such as DH5α and TOP10. Buffer KB is necessary for *endA*+ strains such as HB101, JM110, JM101 and their derived strains.

10. Add **500 µL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **tube**, shaking for 1 minute, discard the supernatant. Put the tbue back to the Magnet.

11. Repeat step **10**.

12. Dry the beads at RT. Tube can also be dried at room temperature for 10~15 minutes.

13. Add 100µl-200ul pre-heat(65℃) Water or Buffer TE to the tube, and then incubate for 5 minutes at 37°C. Vortex or shake the tube for ~120 seconds after incubating for 5 minutes at 37°C to fully elute the plasmid from the beads.

14. Place the tube on an Magnet and allow beads to separate for 2 minutes. With the plate on the Magnet Plate, perform the aspiration, and then transfer the supernatant into a new plate.

15. The DNA concentration can be calculated as follows,

   Concentration (µg/mL)=OD_{260}×50×dilution factor.

Applications: Allsheng Auto-Pure 32A

1. Take 1-5 mL of bacterial culture and follow the previous steps 3-6 to obtain the lysate supernatant.
2. Take a 96-well deep-well plate and add samples and reagents to the plate according to Table 1 below. If the kit is not pre-installed, the following reagents need to be added by yourself.(The total volume of each well must not exceed 1,000 μL,)

Table 1 plate set-up

96-well plate No.	Well position	Sample / reagent	Vol. (μL)	Kit description	Note
Binding	1/7	lysate supernatant	500	Added by user	Try to take the clear lysate, and then add buffer RET and 100% ethanol in turn to get the mixture.
		100% isopropanol	200	Added by user	
		Buffer RET	250	The reagent has been added, no need for user to add	
Beads	2/8	Plasmid-L Beads	35	The reagent has been added, no need for user to add	/
		ddH2O/Elution buffer	65		
Wash 1	3/9	Buffer KB	500	The reagent has been added, no need for user to add	/
Wash 2	4/10	DNA Wash Buffer	600	The reagent has been added, no need for user to add	/
Wash 3	5/11	DNA Wash Buffer	600	The reagent has been added, no need for user to add	/
Elution	6/12	Endofree Elution Buffer	100	The reagent has been added, no need for user to add	Elution volume can be adjusted according to specific requirements, at least 60ul.

3. Start the instrument, place new clean magnetic bar sleeve in the instrument, and put 96-well plates into the corresponding position in the instrument, corresponding to the magnetic bar sleeve.
4. Use the program (table 2).
5. Collect products after the program is completed. Take out 96-well plate, and pipette the product in 6/12 rows of Elution wells into sterile EP tube, and store at -20℃ or -80℃.

Table 2. Extraction procedures

step	name	Well position	Mix time (min)	Magnetize time (s)	Wait time (min)	Vol (μL)	Mix speed (1-10)	Tm (℃)	Mix position (0-100%)	Mix range (1-100%)	Magnetize position (0-100%)	Magnetize speed (1-10)
1	Mix	1	0.2	-	-	900	5	OFF		80		
2	Beads	2	0	10	0	100	-	OFF	0	80	0	1
3	Binding	1	5	60	0	900	4	OFF	0	80	0	1
4	Wash1	3	1	30	0	500	7	OFF	0	80	0	1
5	Wash2	4	1	30	0	600	7	OFF	0	80	0	1
6	Wash3	5	1	30	0.5	600	7	OFF	0	80	0	1
7	Elute	6	5	60	0	100	6	65	0	80	0	1
8	Drop	5	0.5			600	5	OFF		80		

Note: Set to heat up and then action, cooling action synchronization; magnetize mode: magnetize in 4 stages; drying position: upper part of the kit.

Applications: Allsheng Auto-Pure96A

1. Take 1-5 mL of bacterial culture and follow the previous steps 3-6 to obtain the lysate supernatant.
2. Take six 96-well deep-well plates and add samples and reagents to the plate according to Table 1 below. If the kit is not pre-installed, the following reagents need to be added by yourself.(The total volume of each well must not exceed 1,000 μL,)

Table 1 plate set-up

96-well plate No.	Board position	Sample / reagent	Vol. (μL)	Kit description	Note
Binding	1	lysate supernatant	500	Added by user	Try to take the clear lysate, and then add buffer RET and 100% ethanol in turn to get the mixture.
		100% isopropanol	200	Added by user	
		Buffer RET	250	The reagent has been added, no need for user to add	
Beads	3	Plasmid-L Beads	35	The reagent has been added, no need for user to add	/
		ddH2O/Elution buffer	65		
Wash 1	4	Buffer KB	500	The reagent has been added, no need for user to add	/
Wash 2	5	DNA Wash Buffer	600	The reagent has been added, no need for user to add	/
Wash 3	6	DNA Wash Buffer	600	The reagent has been added, no need for user to add	/
Elution	8	Endofree Elution Buffer	100	The reagent has been added, no need for user to add	Elution volume can be adjusted according to specific requirements, at least 60ul.

3. Start the instrument, place new clean magnetic bar sleeve in the instrument, and put 96-well plates into the corresponding position in the instrument, corresponding to the magnetic bar sleeve.
4. Use the program (table 2).
5. Collect products after the program is completed. Take out 96-well plate, and pipette the product in 6/12 rows of Elution wells into sterile EP tube, and store at -20℃ or -80℃.

Table 2. Extraction procedures

step	name	Plate position	Mix time (min)	Magnetize time (s)	Wait time (min)	Vol (μL)	Mix speed (1-10)	Tm (℃)	Mix position (0-100%)	Mix range (1-100%)	Magnetize position (0-100%)	Magnetize speed (1-10)
1	Load	3	-	-	-	-	-	-	-	-	-	-
2	Beads	3	0	80	0	100	-	OFF	1	2	1	20
3	Binding	1	5	80	0	1000	3	OFF	3	2	1	15
4	Wash1	4	0.5	80	0	500	5	OFF	3	2	1	10
5	Wash2	5	0.5	80	0	600	5	OFF	3	2	1	10
6	Wash3	6	0.5	80	2	600	5	OFF	3	2	1	10
7	Elution	8	5	80	0	100	3	65	4	2	1	30
8	Drop	3	0.2			750	6					
9	Unload	3	-	-	-	-	-	-	-	-	-	-

Note: Set to heat up and then action, cooling action synchronization; magnetize mode: magnetize in 4 stages; drying position: upper part of the kit.

Purification of Low-Copy-Number Plasmid/Cosmid

The yield of low copy number plasmid is normally around 0.1-1 μg/mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

✪Culture volume: Use **2×volumes** of the high copy number culture.

✪Use **2×volumes** of the **Buffer A1, Buffer B1, Buffer N3 and Buffer RET**. Additional buffers can be purchased from Biomiga.

✪Use **same volume** of **DNA Wash Buffer** and **EndoFree Elution Buffer**.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low yield	Poor cell lysis.	Resuspend pellet throughly by vortexing and pipetting prior to adding Buffer B1.
		Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1 : 0.2 M NaOH and 1% SDS).
Low yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20°C if the culture is not purified the same day. Do not store culture at 4°C overnight.
Low yield	Low copy number plasmid.	Increase culture volume and the volume of Buffer A1, B1, N3 and RET as instructed on page 9.
No DNA	Plasmid lost in host *E. coli*.	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding Buffer B1.	Do not vortex or mix aggressively after adding Buffer B1. Do not incubate more than 5 minutes after adding Buffer B1.
RNA contamination	RNase A not added to Buffer A1.	Add RNase A to Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel	Ethanol traces were not completely removed from column.	Make sure that no ethanol residue remains in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.

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.beiwobiomedical.com