EasyPrep™ 96-Well Plasmid Miniprep Manual

Catalog# PD96-01, PD96-02

Bioland

If crystals form in buffers, warm at 37 °C to dissolve before use

For research use only

(October 2020)

Contents

Introduction

The Easyprep™ 96-well Plasmid Miniprep kit provides an easy and fast method for isolating high quality plasmid DNA in a high through put format. The key to this system is that the DNA columns avidly but reversibly binds DNA under optimized buffer conditions, while proteins and other unwanted cellular components are removed by wash buffer. High quality plasmid DNAs are collected for downstream applications. By using the 96-well kit, up to 96 samples can be simultaneously processed in less than 90 min. The lysate clearance plate obviates the time-consuming centrifugation step and increases the DNA recovery up to 20%.

Important Notes

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 3 to 5 times. Reference the table below for the commonly used plasmids:

Plasmid	Origin	Copy Numbers	Expected Yield (µg /200 mL)
pSC101	pSC101	5	10-15
pACYC	P15A	10-12	20-25
pSuperCos	pMA2	10-20	20-40
pBR322	pMA2	15-20	30-40
pGEM^{R}	Muted pMA2	300-400	400-500
pBluescript^{R}	ColE1	300-500	400-600
pUC	Muted pMA2	500-700	600-1200

Host strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10, DH5a, and C600 yield high-quality plasmid DNA. *endA*$^{+}$ strains such as JM101, JM110, HA201, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend to transform plasmid to an *endA*$^{-}$ strain if the yield is not satisfactory.

EndA^{-} Strains of E. Coli

BJ5182; DH5α; DH1; DH10B; DH20; DH21; JM103; JM105; JM106; JM107; JM108; JM109; MM294; Select96™; SK1590; SK1592; SK2267; SRB; Stbl2™; Stbl4™; XL1-Blue; XL10-Gold; XLO; TOP10

EndA^{+} Strains of E. Coli

ABLE®C; ABLE®K; BL21(DE3); BMH71-18; C600; CJ236; DH12S™; ES1301; HA201; HMS174; JM83; JM101; JM110; KW251; LE392; M1061; P2392; pLysS; PR700; Q358; RR1; TA2; TG1; TKA2; All NM and Y strains

Optimal cell mass (OD_{600} x mL of Culture): The kit is designed for isolating plasmid grown in standard LB medium for 12 -16 hours to a density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2xYT 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. For over amount of cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, A2 and C1.

Storage and Stability

Buffer A1 should be stored at 4°C once RNase A is added. All other materials can be stored at room temperature. The Guaranteed shelf life is 18 months from the date of purchase.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

- **RNase A**: Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use.
- **Buffer A2** precipitates below room temperature, it is critical to warm up the buffer at 37°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer A2 after use.
- Carry out all centrifugations at **room temperature.**
- DNA Wash Buffer concentrate, add ethanol before use:
  PD96-01:Add 280ml 96-100% ethanol to each bottle.
  PD96-02: Add 400ml 96-100% ethanol to each bottle.

Kit Contents

Catalog#	PD96-01	PD96-02
96-Well DNA Plate	4	20
2.0ml 96-Well Deep Well Plate (Sterile, for bacteria culture)	4	20
1ml 96-Well Deep Well Plate	6	25
96-Well U-Bottom Cell Culture Plate (for Collection)	4	20
Sealing Film	16	80
Buffer A1	110 mL	2x250 mL
Buffer A2	110 mL	2x250 mL
Buffer N1	140 mL	2x350 mL
DNA Wash Buffer concentrate (5x)	2x70mL	6×100 mL
Buffer KB	140 mL	2x350 mL
Column Buffer	50 mL	250 mL
Elution Buffer	50 mL	250 mL
RNase A	370 µL	2x0.85 mL
Use Manual	1	1

Materials supplied by user

- Robotic station if using automated isolation.
- Centrifuge with swing-bucket rotor (3,000 x g).
- Vacuum pump capable of achieving 300-400 mbar if use Vacuum manifold method.
- Standard vacuum manifold.
- Oven or incubator preset to 70°C.

Vacuum Manifold Protocol

1. **Culture and harvest bacteria: Inoculate *E. coli* carrying desired plasmid into 1.0-1.2 ml LB medium in a 2 ml 96-well deep well plate. Grow at 37°C with agitation (180-300 rpm) for 18-20 h.** *The deep well plate doesn't have a cover, use 96-well cell culture plate cover instead. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α™ and JM109™.*

2. **Seal the plate with a sealing film. Pellet bacteria by centrifugation at 1,500-2,000 x g for 5 min in a swing bucket rotor at room temperature.**

3. **Remove the sealing film and discard supernatant. Tapping the inverted plate on a stack of paper towels to remove excess medium. Resuspend bacterial pellet in each well with 200 µl Buffer A1 by pipeting or vortexing** (Seal the plate with sealing film if by vortexing). *Ensure that RNase A has been added to Buffer A1. Complete resuspension of cell pellet is vital for obtaining good yields.*

4. **Add 200 µl Buffer A2 to each well, seal the plate with sealing film, mix thoroughly by inverting 6 times. The solution should become viscous and slightly clear.** *Avoid vigorous mixing as this will shear chromosomal DNA and cause genomic DNA contamination.*

5. **Remove the sealing tape, add 280 µl Buffer N1. Wipe off any buffer residues on the top of the plate and seal the plate with sealing film. Mix by inverting the plate for 6-10 times. A flocculent white precipitate forms.** *Make sure the plate is tightly sealed to prevent cross contamination.*

6. **Centrifuge the plate at 3,000 x g for 10-15 min.** *Optional:* Add 100µl Column Buffer to the center of each well on the DNA plate, wait for 2 minutes, apply the vacuum to drain the column buffer. This column equilibration step recovers the DNA binding capacity when the kit is stored for a while.

7. **Remove the sealing film, transfer the clear lysate into each well of the 96-well DNA plate. Apply vacuum till all the lysate passes through the 96-well DNA plate.**

8. *Optional:* **Add 300 µl Buffer KB to each well and apply vacuum till all the liquid passes through the DNA plate. Turn off the vacuum.** *Buffer KB is required when the plasmid is being isolated from endA$^+$ strains such as TG1, JM110, and HA201. It is not necessary if the plasmid is being purified from endA$^-$ strains such as Top 10 or DH5a. Skip this step if using endA$^-$ strains.*

9. **Add 600 µl DNA Wash Buffer to each well and turn on vacuum till all Buffer passes through the plate. Turn off vacuum. Repeat once.** *Ensure that the ethanol is added to DNA Wash Buffer before use.*

10. **Discard the waste in the manifold and dry the DNA plate with maximum vacuum power for 20 min.**

11. **Remove the DNA plate from the manifold and tap the plate on a stack of absorbent paper towels. Remove any residual moisture from the tip ends of the DNA plate with clean paper towel.**

12. *Optional:* **Place the DNA plate into a vacuum oven preset at 70°C for 10 min.**

13. **Place the DNA plate back to the vacuum manifold and apply maximum vacuum for another 5 min.** *It's critical to remove the residual ethanol from the membrane.*

14. **Place a new 0.5ml deep-well-plate or UV transparent plate inside the manifold with a manifold adaptor. Place the DNA plate on top of it.**

15. **Add 100 µl Elution Buffer (or sterile water) to each well, let the plate stand for 2 min. Apply maximun vacuum for 5–10 min to elute the DNA. Turn off vacuum and ventilate the manifold slowly.** *Note: The DNA yield depends on the elution volume. Use less elution Buffer if higher DNA concentration is desired.*

Centrifuge Protocol

1. **Culture and harvest bacteria: Inoculate *E. coli* carrying desired plasmid into 1.0-1.2 ml LB medium in a 2ml 96-well deep well plate. Grow at 37°C with agitation (180-300 rpm) for 18-20 h.** *The deep well plate doesn't have a cover, use 96-well cell culture plate cover instead. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α™ and JM109™.*

2. **Seal the plate with sealing film. Pellet bacteria by centrifugation at 1,500-2,000 x g for 5 min in a swing bucket rotor at room temperature.**

3. **Remove the sealing film and discard supernatant. Tapping the inverted plate on a stack of paper towels to remove excess medium. Resuspend bacterial pellet in each well with 200 µl Buffer A1 by pipeting or vortexing** (Seal the plate with sealing film if by vortexing). *Ensure that RNase A has been added to Buffer A1. Complete resuspension of cell pellet is vital for obtaining good yields.*

4. **Add 200 µl Buffer A2 to each well, seal the plate with sealing film, mix thoroughly by inverting 6 times. The solution should become viscous and slightly clear.** *Avoid vigorous mixing as this will shear chromosomal DNA and cause genomic DNA contamination.*

5. **Remove the sealing tape, add 280 µl Buffer N1. Wipe off any buffer residues on the top of the plate and seal the plate with sealing film. Mix by inverting the plate for 6-10 times. A flocculent white precipitate forms.** *Make sure the plate is tightly sealed to prevent cross contamination.*

6. **Centrifuge the plate at 3,000 x g for 10-15 min.** *Optional:* Add 100µl Column Buffer to the center of each well on the DNA plate, wait for 2 minutes, Place the DNA plate on top of a 0.5ml deep-well-plate in a swing bucket rotor and centrifuge at 3000 x g for 5 min. Discard the flow through liquid and reuse the deep

well-plate. This column equilibration step recovers the DNA binding capacity when the kit is stored for a while.

7. **Remove the sealing film, transfer the clear lysate into each well of the 96-well DNA plate.**

8. **Place the DNA plate on top of a 0.5ml deep-well-plate in a swing bucket rotor and centrifuge at 3000 x g for 5 min. Discard the flow through liquid and reuse the deep-well-plate for next step.**

9. *Optional:* **Add 300 µl Buffer KB to each well and centrifuge at 3000 x g for 5 min. Discard the flow-through liquid and reuse the deep-well-plate for next step.** *Note: Buffer KB is required when the plasmid is being isolated from endA$^+$ strains such as TG1, JM110, and HA201. It is not necessary if using endA$^-$ strains.*

10. **Add 600 µl DNA Wash Buffer to each well and centrifuge at 3000 x g for 5 min. Discard the flow through liquid and put the DNA plate back to the deep-well-plate.**

11. **Repeat once.**

12. **Centrifuge the 96-well DNA plate at 3,000 x g for 20 min.** *It's critical to remove the residual ethanol from the membrane. Rinse the 0.5ml deep-well-plate with water and reuse for next prep.*

13. *Optional:* **Place the DNA plate into a vacuum oven preset at 70°C for 10 min.**

14. **Place the DNA plate on top of a new 0.5ml deep-well-plate or an UV transparent plate. Add 100 µl Elution Buffer to each well of the DNA plate. Let the plate stand for 2 min.**

15. **Centrifuge the plate at 3000 x g for 5 min to elute the DNA. Note: The DNA recovery rate and concentration depend on the elution volume. For maximum yields, elute with another 100 µl Elution Buffer although the DNA concentration will be lower.**

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA Yields	Poor cell lysis	Do not use more than 2 ml of overnight culture. Resuspend the cell pellet completely. Buffer A2 bottle needs to be tightly capped.
	Bacterial overgrown	Do not grow Bacterial culture for more than 16 hours.
	Culture not fresh	Use fresh culture or the bacteria pellet at -20°C or -80°C. Do not storage the culture at 4°C.
	Low copy number plasmid	Scale up culture volume and Buffer volume accordingly.
No DNA eluted	Forget to add ethanol to the DNA wash Buffer	Prepare the DNA Wash Buffer as instructed.
Chromosomal DNA contamination	Over mixing after adding Buffer A2	Do not vortexing or vigorously mixing after Buffer A2 is added.
OD doesn't match the DNA yield or DNA flow out of agarose gel during loading	Trace ethanol contamination	Wash the plate as instructed
RNA visible on agarose gel	Forget to add the RNase A to Buffer A1	Add RNase A to Buffer A1 before use.
Lysate clearance plate clogged	Lysate was not mixed well after adding Buffer N1	Mix the lysate well by inverting the plate for 5 times and then vortex for 10 seconds.

Related products

Catalog #	Product Name	Preps
PD01-01	Plasmid Miniprep kit	50
PD01-02	Plasmid Miniprep kit	250
PD02-01	Plasmid Miniprep-II kit	50
PD02-02	Plasmid Miniprep-II kit	250
PD03-01	Plasmid Midiprep kit	10
PD03-02	Plasmid Midiprep kit	25
PD04-01	Plasmid Midiprep-II kit	10
PD04-02	Plasmid Midiprep-II kit	25
PD03-11	Plasmid ezFilter Midiprep kit	10
PD03-12	Plasmid ezFilter Midiprep kit	25
PD04-11	Plasmid ezFilter Midiprep-II kit	10
PD04-12	Plasmid ezFilter Midiprep-II kit	25
PD05-01	Plasmid Maxiprep kit	10
PD05-02	Plasmid Maxiprep kit	25
PD05-11	Plasmid ezFilter Maxiprep kit	10
PD05-12	Plasmid ezFilter Maxiprep kit	25
PD03-21	EndoFree Plasmid Midiprep kit	10
PD03-22	EndoFree Plasmid Midiprep kit	25
PD03-31	EndoFree Plasmid ezFilter Midiprep kit	10
PD03-32	EndoFree Plasmid ezFilter Midiprep kit	25
PD04-21	EndoFree Plasmid Midiprep-II kit	10
PD04-22	EndoFree Plasmid Midiprep-II kit	25
PD04-31	EndoFree Plasmid ezFilter Midiprep-II kit	10
PD04-32	EndoFree Plasmid ezFilter Midiprep-II kit	25
PD05-21	EndoFree Plasmid Maxiprep kit	10
PD05-22	EndoFree Plasmid Maxiprep kit	25
PD05-31	EndoFree Plasmid ezFilter Maxiprep kit	10
PD05-32	EndoFree Plasmid ezFilter Maxiprep kit	25

Bioland Scientific LLC

14925 Paramount Blvd., Suite C

Paramount, CA 90723

USA

Tel: (562) 602-8882

Fax: (562) 733-6008

Email: service@bioland-sci.com

order@bioland-sci.com

Visit our web at **www.bioland-sci.com** and learn more about Bioland products