pDNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi pDNA Miniprep Kit is designed to have a fast, easy and economical isolation of upto 20µg of high purity pDNA from bacterial cultures with both high copy as well as low copy numbers. The kit incorporates a uniquely formulated buffer AL2 to lyse the bacterial culture and fast spin column technology to purify it. This technology does away with phenol extraction (associated with desalting) and ethanol precipitation (associated with anion exchange based purification).

2. Applications

1. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Restriction Digestion
4. Cloning
5. PCR

3. Storage Conditions

mdi pDNA Miniprep Kit should be stored at room temperature. The kit is stable for one year at above storage conditions without showing any reduction in performance and quality.

For longer storage, the entire kit can be stored at 2-8°C. In case precipitates are observed in buffer, re-disolve all buffers before use at 37°C for few minutes. All buffers should be at room temperature before starting the protocol.

4. Quality Assurance

The **mdi** pDNA Miniprep kit is designed for various pre-determined specifications and user requirements such as yield, purity, ruggedness, shelf life and functional convenience.

These are produced through a well defined quality management system certified by Underwriters Laboratories, USA for ISO 9001: 2008 which ensures intra lot as well as lot to lot consistency.

5. Safety Information

The buffers and the reagents may contain irritants, so wear lab coat, disposable gloves and protective goggles while working with the pDNA Miniprep Kit.

6. Lot Release Criteria

Each lot of pDNA Miniprep Kit is tested against predetermined specifications to ensure consistent product quality.

7. Technical Support

At **mdi**, customers are our priority. We will share our experiences to assist you to overcome problems in general product usage as well as customize products for special applications. We will

* Stimulate problems, and suggest alternative methods to solve them.
* Make changes/ improvements in our existing products/protocols.
* Develop special new products and system especially to satisfy your needs.

We welcome your feedback to improve our products.

8. Kit Contents

Contents	Quantity			Storage Temperature
Spin Columns	50	250	1000	RT
Buffer AL1	20ml	100ml	400ml	RT
Buffer AL2	20ml	100ml	400ml	RT
Buffer AL3	30ml	150ml	600ml	RT
Buffer W	60ml	300ml	1200ml	RT
Buffer E	15ml	75ml	300ml	RT
RNaseA (2.3 units/µl)	50µl	250µl	1000µl	2-8°C
Collection Tubes	50	250	1000	RT
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

9. Specifications

pDNA Binding Capacity	≥25µg
Capacity of column reservoir	800µl
Binding capacity of membrane (ds DNA)	20µg
Recovery	80%
Minimum elution volume	50µl
Total time taken	25 minutes

10. Volumes for a Miniprep

Culture volume	1-5ml
Buffer AL 1	250µl
Buffer AL 2	250µl
Buffer AL 3	350µl
Buffer W	750µl
Buffer E	50µl

Note: For low copy plasmids use 3-5ml culture volume

11. Principle

Obtaining highly pure pDNA using **mdi** pDNA Miniprep Kit involves: Lysis and neutralization of bacterial culture, Capturing pDNA on to spin column, Washing and Elution of pDNA to get ultrapure concentrated pDNA.

1. Lysis and neutralization of bacterial culture

For efficient lysis of bacterial culture, centrifugation and resuspension steps must be performed with buffer having optimal salt concentration and pH conditions according to Birnboim and Doly. Excessive lysis can lead to denatured DNA. For the preparation of lysate refer page no. 12.

2. Capturing pDNA on to spin column

In order to facilitate adsorption of pDNA on to the spin column, suitable conditions of salt concentration and pH are achieved by addition of buffer AL3.

3. Washing

Subsequent to pDNA adsorption, RNA, proteins, dyes and low molecular weight impurities are washed away. Washing is done with buffer W.

4. Elution of pDNA to get ultrapure concentrated pDNA

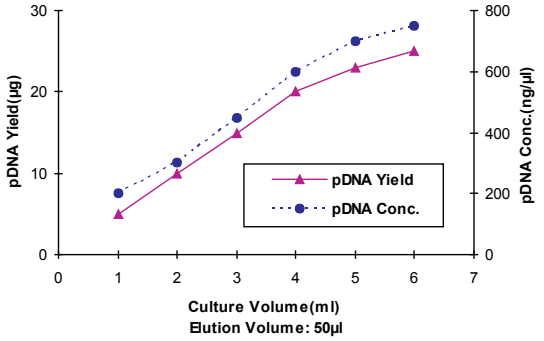
Salt concentration and pH of elution buffer is very important for elution efficiency. Elution occurs in basic concentration and at low salt concentration. Maximum elution efficiency is achieved at pH 7.5-8.5 Elution is done with 50µl of buffer E to provide highly concentrated ultrapure pDNA.

5. Yield

To ensure optimum yield, apply elution buffer to the center of column membrane. Other important factors to be considered are:

1. Culture volume
2. Elution Volume
3. Incubation Time with Elution Buffer

Culture Volume vs. pDNA Yield and Concentration



Test Conditions

Host cell: $DH_5\alpha$

Plasmid: pUC

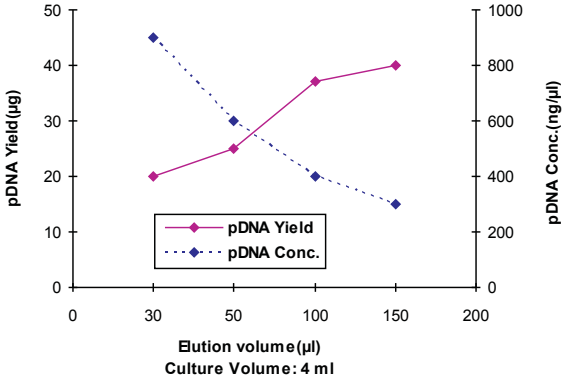
Fragment size: 9 Kb

Copy number: 500-700

Elution volume: 50µl

Culture volume: 1-6ml

Elution Volume vs. pDNA Yield and Concentration



Test Conditions

Host cell: $DH_5\alpha$

Plasmid: pUC

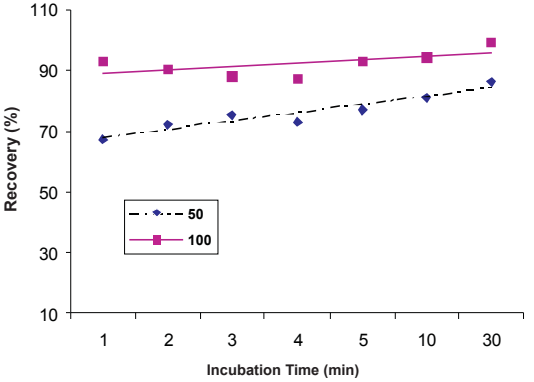
Fragment size: 9 Kb

Copy number: 500-700

Elution volume: 50µl

Culture volume: 4ml

Incubation Time vs. Recovery



Test Conditions

Host cell: $DH_5\alpha$

Plasmid: pUC

Fragment size: 9 Kb

Copy number:500-700

Elution volume:50µl

Culture volume:3ml

12. How to Begin

1. Growing and Culturing Bacteria

A. Essential bacterial culture media and antibiotic conditions

a. For growth and propagation of bacterial cells, high quality LB-medium is used.

b. Screening of bacteria is done by the addition of suitable selective markers like antibiotics.

c. For high yields and superior quality, various factors like plasmid copy number, host strains, inoculation, antibiotics and type of culture medium are taken into account.

B. Plasmid Copy Number

a. Plasmids can differ widely in their copy number per cell, depending on their origin of replication which entirely determines their state of control (whether relaxed or stringent) and depends on the size of insert.

b. For attaining high copy numbers within the bacterial cells, the plasmids such as pUC series and their derivatives undergo mutations.

Plasmids like pBR-322 and its derivatives are present in lower copy numbers.

Large cosmids and plasmids are often maintained at very low copy per cell.

Origins of Replication and Copy Numbers of Various Plasmids

DNA Construct	Origin of Replication	Copy Number	Classification
Plasmids			
pUC Vectors	pMB1*	500-700	High Copy
pBluescript® Vectors	Col E1	300-500	High Copy
pGEM® Vectors	pMB1*	300-400	High Copy
pTZ Vectors	pMB1*	>1000	High Copy
pBR322 and Derivatives	pMB1*	15-20	Low Copy
pACYC and Derivatives	p15A	10-12	Low Copy
pSC101 and Derivatives	pSC101	~ 5	Very Low Copy
Cosmids			
SuperCos	Col E1	10-20	Low Copy
pWE15	Col E1	10-20	Low Copy

* The pMB1 origin of replication is closely related to that of Col E1 and falls in the same incompatibility group. The high copy number plasmids listed here contain mutated versions of this origin.

C. Host Strains

The following table will help select the most suitable strain:

Strains Used	Applications
DH1 & C600	High quality DNA
XL1-Blue	High quality DNA suitable for sequencing. Reproducible and reliable results.
$DH_5\alpha$	High quality DNA Reproducible and reliable results

Do's:

Also consider methylation and growth characteristics of the host strain during selection.

Don't's

Strain HB101 and its derivatives such as TG-1 and JM series are not recommended, as these produce large amounts of carbohydrates which are released during lysis and may inhibit enzyme activities if not completely removed. In addition; they exhibit endonuclease activity leading to reduction in DNA quality.

D. Inoculation

Do's

1. For plasmid preparation; bacterial cultures should always grow from a single colony picked from a freshly streaked plate.
2. For isolating desired clones; streaking from a glycerol stock should be done onto a freshly prepared agar plate, containing the suitable selective marker.
3. For obtaining a single colony of an antibiotic resistant clone, procedure should be repeated.

Don'ts

Poor microbiological practices such as subculturing directly from glycerol stocks, agar stabs, liquid cultures and inoculation from plates that have been stored for a long time lead to loss or mutation of plasmid.

E. Growth conditions

Do's

1-5 ml culture media containing suitable selective agent should be used for inoculating this single colony followed by vigorous shaking for 12-16 hours.

Don'ts

Using cultures grown for more than 16 hours can lead to inefficient lysis and reduced plasmid yields.

F. Selection of antibiotics

Most of the commonly used plasmids do not have Par Locus which ensures their equal segregation during cell division. The resulting daughter cells which do not contain plasmids replicate more rapidly and take over the culture ,which may result in poor plasmid yields.

Antibiotics are used to supress the growth of such cells that do not have plasmids. On the other hand, some of the antibiotic resistant clones have plasmid linked bla gene which produces Beta-lactamase which in turn hydrolyses Ampicillin, a commonly used antibiotic.

Some of the commonly used antibiotics with their working concentrations and storage conditions are shown in table below:-

Antibiotic	Stock solutions		Working concentration (dilution)
	Concentration	Storage	
Ampicillin(sodium salt)	50 mg/ml in H_2O	-20°C	100µg/ml(1/500)
Chloramphenicol	34 mg/ml in ethanol	-20°C	170µg/ml(1/200)
Kanamycin	10 mg/ml in H_2O	-20°C	50µg/ml(1/200)
Streptomycin	10 mg/ml in H_2O	-20°C	50µg/ml(1/200)
Tetracycline HCl	5 mg/ml in ethanol	-20°C	50µg/ml(1/100)

G. Culture Media Selection

Do's

Recommended cell density and culture O.D

1. The recommended optical density should vary from 2.5-3.5.
2. Luria-Bertani(LB) broth is the recommended culture medium for use with **mdi** kits.
3. We recommend growing cultures in LB medium containing 10g NaCl,10g tryptone and 5g yeast extract per liter to obtain high yields with **mdi** columns.
4. Use reduced culture volumes if richer broths are used. Optimal cell density determines amount of culture volume to be used.

Don'ts

1. Richer broths such as TB or 2x YT and rapidly growing strains can lead to extremely high cell densities which result in overloading of the purification columns and high viscosity.
2. Too much culture volume leads to inefficient alkaline lysis which result in overloading of **mdi** membrane and poor performance of the column.
3. Shearing of bacterial gDNA and contamination of pDNA may take place if medium has excessive viscosity which would then require vigorous mixing.

2. Preparing Cell Lysates

A. Bacterial lysis

Do's

1. Bacterial lysis is performed under alkaline conditions. After harvesting and resuspension , the bacterial cells are lysed in NaOH/SDS. SDS is an anionic detergent which solublizes the phospholipid and protein components of the cell membrane and alkaline conditions denature the chromosomal plasmid DNA's as well as proteins.
2. For maximum pDNA yields without any gDNA contamination, optimum lysis time should be used. Prolonged exposure of plasmid to (alkaline) lysis conditions can irreversibly denature it. This denatured plasmid runs faster on agarose gel and is resistant to Restriction Enzyme digestion.
3. Mixing of buffers should be slow and by gentle inversion.

Don'ts

Vigorous treatment during the cell lysis will lead to shearing of the bacterial chromosome due to which free chromosomal DNA fragments are left behind in the supernatant. Since chromosomal fragments are chemically indistinguishable from pDNA under the conditions used, the two species will not be separated on **mdi** membrane and will elute under the same low salt conditions.

B. Neutralisation of cell lysates

1. To efficiently neutralise the lysates, high salt conditions are maintained by the addition of buffer 'AL3'. High salt concentration results in denatured proteins, chromosomal DNA, cellular debris and SDS precipitates.
2. Complete and gentle mixing of solution is necessary to ensure precipitation.

3. Agarose Gel Electrophoresis

Agarose gel electrophoresis can be used to analyse the pDNA isolation procedure.

Samples from cleared lysate and flow through are compared with the eluate. Clear lysate and flow through samples are precipitated with isopropanol and resuspended in a minimal amount of TE (Tris. EDTA) before loading into the gel. The photograph below shows the comparison.

Agarose Gel Analysis showing a step by step pDNA isolation using **mdi** pDNA Miniprep Kit.

* **Lysate:** Closed circular pDNA and degraded RNaseA resistant RNA.
* **Flow through:** Only degraded RNA and no pDNA.
* **Eluate:** Pure pDNA without any contamination with other nucleic acids.

13. Important Points to be Considered

Buffer

1. All buffers should be stored at room temperature.
2. After adding RNase A to buffer AL1, store it at 2-8°C.
3. For Complete neutralization, mix the contents by inverting 2-3 times more.

Centrifugation

1. All centrifugation steps should be performed at ≥ 10,000 rpm in conventional table top micro centrifuge
2. In case of choking of spin column, increase centrifugation time.

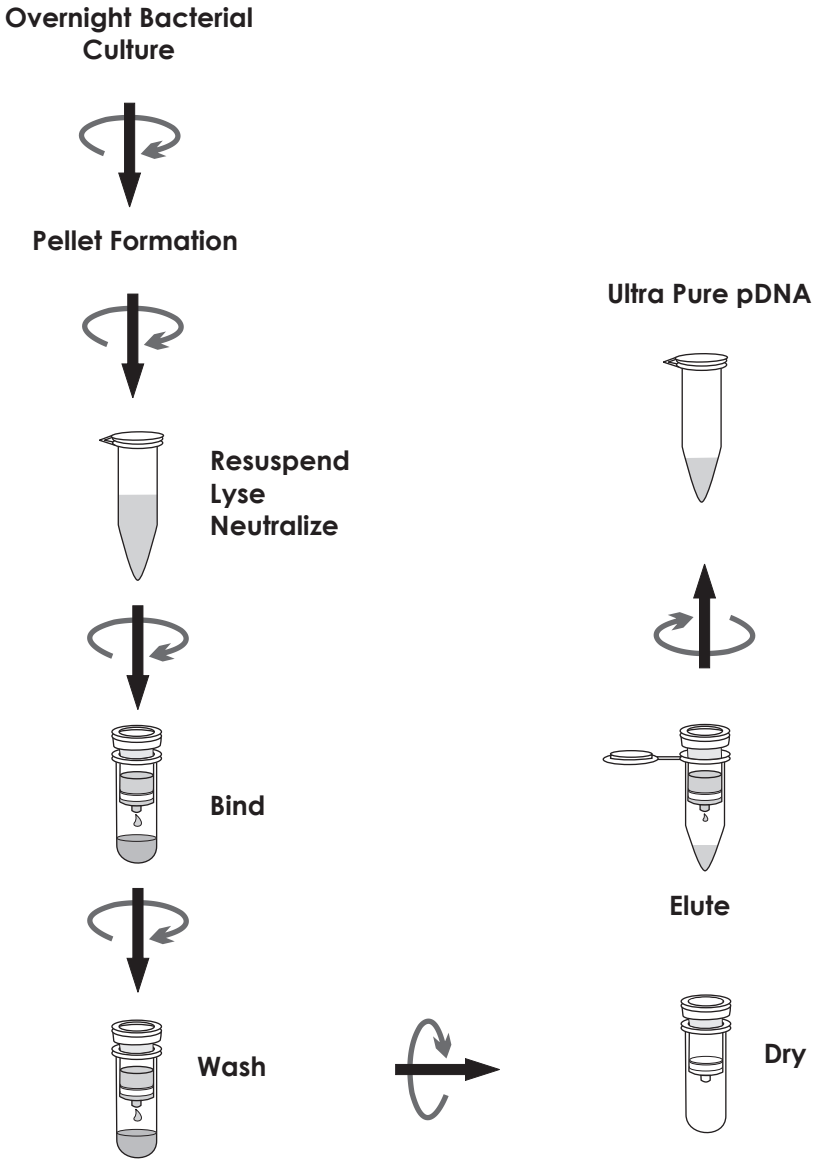
Washing

To remove residual wash buffer spin the column at ≥10,000 rpm for 1 minute, otherwise it will inhibit enzymatic reactions.

Elution

For maximum elution efficiency, dispense the elution buffer on to the center of column membrane.

14. mdi pDNA Miniprep Procedure



15. Protocol

Note: Add provided RNaseA in the buffer AL1 before use by pipetting 1ml AL1 buffer into the RNase A vial, shake it, and then transfer the mixture into the AL1 bottle. Store at 2-8°C and use within 6 months.

***If precipitates are observed in buffer AL2, keep it at 37 °C for some time.**

1. Centrifuge 1-5ml of overnight bacterial culture at 10,000 rpm for 5 minutes. Remove all the traces of supernatant by inverting the open tube until all medium has been drained.

2. Resuspend the pellet in 250µl of buffer AL1 completely by vortexing or pipetting up and down and transfer to a 1.5ml micro centrifuge tube.

   Ensure that RNaseA has been added to buffer AL1.

   No cell clumps should be visible after resuspension.

3. Add 250µl of buffer AL2 , mix gently but thoroughly by inverting the tube 4-6 times.

   Mix gently by inverting the tube .Do not vortex ,as this may result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 2 minutes.

   Bottle containing buffer AL2 should be closed immediately after use to avoid acidification from CO_2 in the air.

4. Add 350 µl of buffer AL3, mix immediately but gently by inverting the tube 4-6 times.

5. Centrifuge at 13,000 rpm for 10 minutes. A compact white pellet will form.

   Note: Increase centrifugation time to 15 minutes at 10,000 rpm

6. Place the spin column in the collection tube. Collect the supernatant quickly and carefully and pass through the spin column by giving a spin at ≥10,000 rpm for 1 minute. Discard the flow through.

   Note: Maximum volume of the column reservoir is 800µl. For sample volumes >800µl, simply load the remaining sample, balance the micro centrifuge and spin again. Discard the flow through.

7. Place the spin column in the same collection tube. Wash the miniprep spin column with 750µl of buffer W by giving a spin at ≥10,000 rpm for 1 minute. Discard the flow through.

8. Place the spin column in same collection tube and centrifuge for an additional 1 minute at ≥10,000rpm.

   Important: This step is necessary to remove residual wash buffer. Residual wash buffer will not be completely removed unless the flow through is discarded before this additional centrifugation. Residual wash buffer may inhibit subsequent enzymatic reactions.

9. Place the spin column in a fresh 1.5ml micro centrifuge tube (not provided).

10. Elute the bound pDNA in a minimum of 50µl of buffer E (users can elute the pDNA in a greater volume if they wish to) by adding the buffer directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥10,000 rpm for 1 minute.

    Note Long term storage :- DNA can be eluted in TE buffer (10 mM trisC1, 1mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

16. Trouble Shooting Guide

A. Little or no DNA

1. Plasmid did not propagate	Please check that the conditions for optimal culture growth were met.
2. Poor bacterial growth	Inoculate from a freshly streaked plate and incubate in a shaker after inoculation.
3. Precipitates in Buffer AL2	Redissolve by warming to 37 °C.
4. Cell resuspension incomplete	The bacterial pellet should be evenly resuspended in buffer AL1. Do not add buffer AL2 until an even suspension is achieved.
5. Poor cell lysis	Too many cells harvested from an overgrown culture. Use culture of proper O.D. grown in not more than 16 hours.
6. Improper dispensing of the elution buffer	The elution buffer must be dispensed properly on to the center of the column membrane.

B: Low quality DNA

1. Nuclease contamination	Use autoclaved plastic and glass wares.
2. RNA contamination	RNase digestion is insufficient. Check that RNase A is added to buffer AL1. Also if buffer AL1 is more than 6 months old, add more RNase A.
3. Genomic DNA in eluate	Avoid excessive vortexing or vigorous mixing.

4. Plasmid degradation	Do not incubate in AL2 for more than prescribed time in step 3.
5. Incomplete drying	Results in solvent in the eluate which is difficult to load in gel, also results in reduced yield and difficulties in digestion. Spin the column for 2-3 minutes extra at ≥10,000 rpm to completely remove the solvent.

17. Product Use Limitations

mdi kits are developed and manufactured for research purpose only. The products are not recommended to be used for human, diagnostics or drug purposes for which these should be cleared by the concerned regulatory bodies in the country of use.

18. Product Warranty and Satisfaction Guarantee

All **mdi** products are guaranteed and are backed by our

a. Technical expertise and experience of over 30 years.

b. Special **mdi** process for consistency and repeatability.

c. Strict quality control and quality assurance regimen.

d. Certificate of analysis accompanied with each product.

mdi provides an unconditional guarantee to replace the kit if it does not perform for any reasons other than misuse. However, the user needs to validate the performance of the kit for its specific use.

19. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	**Code**					**Pack Size**	**Code**
MIPK	MIPK					50	0050
						250	0250
						1000	1000

Example:

MIPK	XX	XX	XX	X	0250

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