Express 96 pDNA Miniprep Kit

User Guide

mdi
Membrane Technologies

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1. Introduction

mdi Express 96 pDNA Miniprep Kit is designed to have a fast, easy and economical isolation of upto 25µg 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 vacuum 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. Transfection/Transformation/Transduction
2. Automated Fluorescent Sequencing
2. Radioactive Sequencing
3. Restriction Digestion
4. Labeling
5. Cloning

3. Storage Conditions

mdi Express 96 pDNA Miniprep Kit should be stored at room temperature. The kit is stable for one year at room temperature 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** Express 96 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 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 Express 96 pDNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Express 96 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
Express Filter Plate	1	RT
Express Prep Plate	1	RT
Rack of Collection Microtubes	1	RT
Flat Bottom Block (Sterile)	1	RT
Caps for Collection Microtube	96	RT
Aluminum Foil (Sterile)	2	RT
Tape Pad (Sterile)	5	RT
Buffer AL1	30ml	RT
Buffer AL2	30ml	RT
Buffer AL3	40ml	RT
Buffer W	200ml	RT
Buffer E	20ml	RT
RNaseA (2.3 Units/µl)	75µl	2-8 °C
Hand Book	1	–
Certificate of Quality	1	–

9. Specifications

pDNA Binding Capacity	≥25µg
Capacity of column reservoir	1ml
Recovery	80%
Minimum elution volume	75µl
Total time taken	45 minutes

10. Volumes for a Miniprep

Culture volume	1-5ml
Buffer AL 1	250µl
Buffer AL 2	250µl
Buffer AL 3	350µl
Buffer W	1800µl
Buffer E	100µl

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

11. Principle

Obtaining highly pure pDNA using **mdi** Express 96 pDNA Miniprep Kit involves: Lysis and neutralization of bacterial culture, Capturing pDNA on to prep plate, Washing and Elution of pDNA to get ultrapure concentrated pDNA.

1. Lysis and neutralization of bacterial culture

For efficient lysis of bacterial culture, resuspension and lysis 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. 11.

2. Capturing pDNA on to prep plate

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

3. Washing

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

4. Elution of pDNA to get ultrapure concentrated pDNA

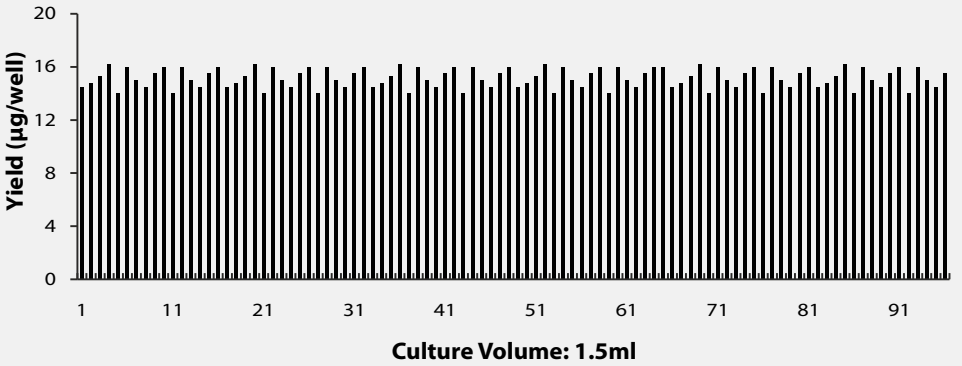
Salt concentration and pH of elution buffer is very important for elution efficiency. Elution occurs in basic conditions and at low salt concentration. Maximum elution efficiency is achieved at pH 7.5-8.5. Elution is done with 100µl of buffer E to provide highly concentrated ultrapure pDNA.

5. Yield

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

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

High Consistency: Well to Well



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 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 be grown 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 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 at 600nm.
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. Growth of bacterial culture in a 96 well flat bottom block for express 96 miniprep pDNA kit.

Fill each well of a 96-well flat-bottom block with 1 ml of growth medium containing the appropriate antibiotic. Inoculate each well from a single bacterial colony. Incubate the cultures for 20–24 hrs at 37°C with vigorous shaking.

The wells in the block may be protected against spill-over by covering the block loosely with a sterile aluminum foil (provided). Loose aluminum foil will promote gas exchange during growth period.

3. 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 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. Neutralization of cell lysates

1. To efficiently neutralize the lysates, high salt concentrations are maintained by the addition of buffer 'AL3'. High salt concentration results in precipitation of denatured proteins, chromosomal DNA, cellular debris and SDS.

2. Complete and gentle mixing of solution is necessary to ensure precipitation.

4. Agarose Gel Electrophoresis

Agarose gel electrophoresis can be used to analyse the Express 96 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** Express 96 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.
4. If precipitates are observed in buffer AL2 keep it at 37°C for some time.

Centrifugation

1. All centrifugation steps should be performed at 2500-3000 rpm in a centrifuge with rotor for microtiter plate.

Washing

To remove residual wash buffer apply maximum vacuum for 10 minutes, otherwise it will inhibit enzymatic reactions.

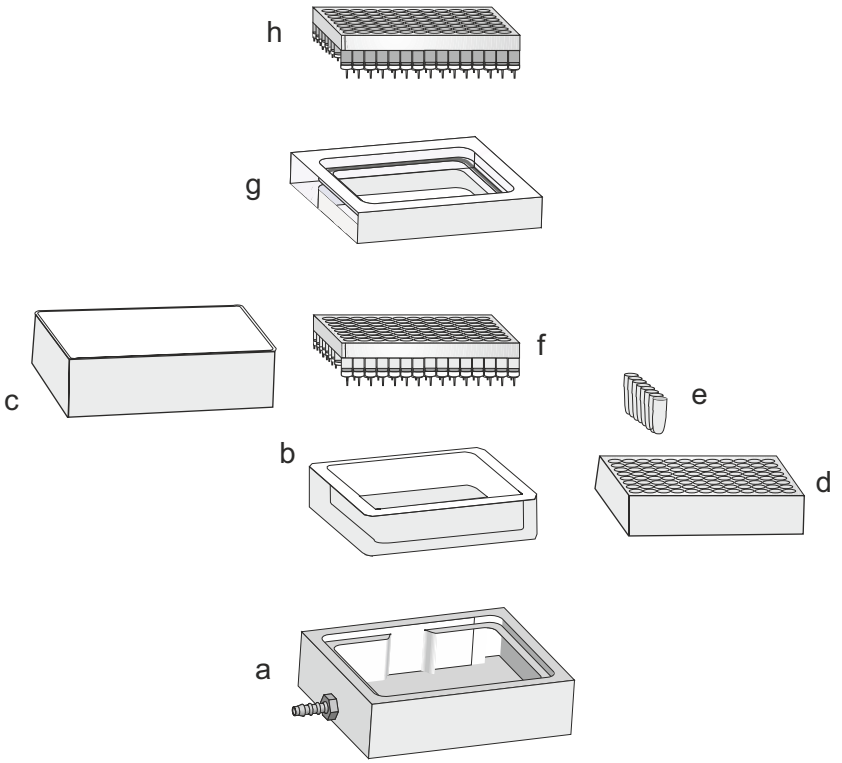
Elution

For maximum elution efficiency, dispense the elution buffer on to the center of well membrane. Average eluate volume is 60µl from an elution buffer volume of 100µl. Apply maximum vacuum during elution.

Vacuum Note:

1. Regulate (-30 to -150) mmHg vacuum on empty module using 3-way valve or vacuum regulator on **mdi** VM 96 before starting the procedure.
2. Switch off vacuum between steps to ensure that a consistent, even vacuum is applied.
3. Wear safety glasses when working near VM 96.
4. Vacuum pressure will increase during procedure.
5. Vacuum pressure will decrease as the sample passes through the wells.

14. mdi Vacuum Manifold for 96 Well Purification Kits (VM 96)



mdi VM 96 Manifold Components

a. mdi VM Base

b. Plate Holder

c. Waste Tray

d. Microtube Rack

e. Microtubes

f. Express Prep Plate

g. mdi VM 96 Top Plate

h. Express Filter Plate

15. Guidelines for mdi vacuum Manifold for 96 Well Purification Kits (VM 96)

1. **mdi** VM 96 manifold is operated with a house vacuum or Vacuum Pump

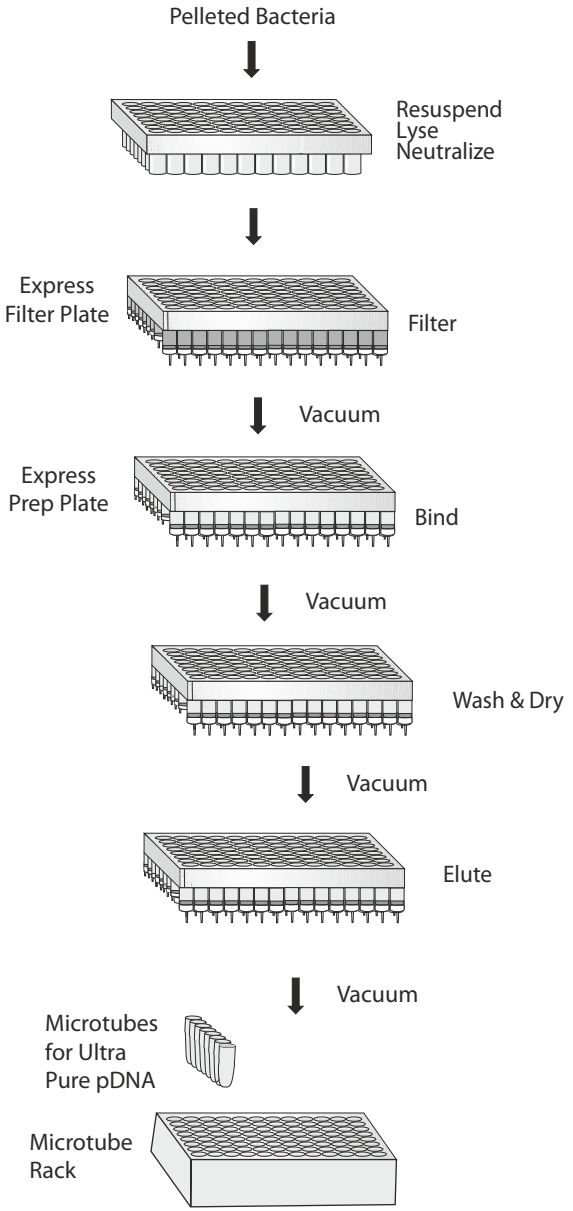
2. Always store **mdi** VM 96 manifolds clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not use solvents.

3. The components of **mdi** VM 96 manifold are not resistant to ethanol, methanol, or other organic solvents. If solvents are spilled on the unit, rinse thoroughly with distilled water. Ensure that no residual Buffer W should be left in the **mdi** VM 96 manifold.

Things to be provided by the User

1. **mdi** VM 96 manifold
2. Multichannel Pipettes
3. Reservoir for multichannel pipettes
4. Centrifuge for micro titer plate
5. Vacuum Source
6. Vacuum Regulator

16. mdi Express 96 pDNA Miniprep Procedure



17. Protocol

This protocol is designed for high-throughput plasmid DNA minipreps using Express filter and express prep plates on **mdi** VM 96. The kit accommodates upto 96 parallel preparation of up to 25 µg of high copy plasmid DNA from 1-5ml overnight cultures of E. coli grown in LB (Luria-Bertani) medium. If 1 ml overnight cultures are used, upto 96 cultures can be grown in a flat-bottom block.

Note:

a. Add RNase A in the buffer AL1 before use by pipetting 0.5ml buffer AL1 into the RNase vial, shake it, and then transfer the mixture into the AL1 bottle. Store at 2-8 °C and use with in 6 months.

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

Prepare mdi VM 96:

c. Place the Express filter plate in the **mdi** VM96 top plate, make sure that the plate is seated securely. Seal unused wells of the Express filter plate with tape.

d. Place the plate holder inside of the **mdi** VM base, place express prep plate in to the plate holder.

e. Place **mdi** VM 96 top plate squarely over base. The express prep plate should now be placed under the express filter plate. Attach **mdi** VM to a vacuum source.

f. Regulate (-30 to -150) mmHg vacuum on empty module using 3 way valve on vacuum regulator on **mdi** VM 96 before starting the procedure.

Procedure:

1. Centrifuge bacterial culture in flat bottom block by centrifuging for 5 minutes at 2500-3000rpm in a centrifuge with rotor for micro titer plate preferably at 4-10°C. The block should be covered by adhesive tape during centrifugation. Remove media by inverting the block.

   To remove media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of medium.

2. Resuspend the pellets with 250µl of buffer AL1 completely. (Transfer the pellet in flat bottom block after resuspension in 250µl of buffer AL1 if cells were not harvested in this block).

3. Add 250µl buffer AL2 to each sample. Dry the top of the flat bottom block with a paper towel, seal the block with the tape provided. Ensure proper sealing by pressing the top of the tape horizontally across the plate with a tissue paper. Gently invert the block 4-6 times to mix.

   It is important to mix gently by inverting the block . Do not shake vigorously, as this will result in shearing of genomic DNA. If necessary continue inverting the block until the solution, becomes viscous and translucent.

4. Centrifuge the flat bottom block for 30 seconds at 2000rpm before removing the tape to ensure that the liquid does not stick to the walls and rim of the wells. This prevents cross contamination between wells.

5. Remove the tape from the block. Add 350µl of buffer AL3 to each sample, dry the top of the flat bottom block with a paper towel, and seal the block with a new tape sheet. Ensure proper sealing by pressing the top of the tape horizontally across the plate with a tissue paper. Gently invert the block 4-6 times to mix.

   To avoid localized precipitation, mix the samples gently but throughly, immediately after addition of buffer AL3. The solution should become cloudy.

6. Now repeat step 4 and then remove the tape from the flat bottom block. Pipet the lysates from flat bottom block into the wells of the express filter plate. Unused wells of the express filter plate should be sealed with tape. Apply vacuum until all samples have passed through.

   The optimal flow rate is approximately 1-2 drops/second which can be regulated by using a 3-way valve or vacuum regulator between the **mdi** VM and the vacuum source.

7. Switch off vacuum and ventilate the **mdi** VM 96 slowly. Discard Express Filter Plate. Put the express prep plate having the cleared lysates to the top plate of the **mdi** VM 96. Seal any unused wells of the express prep plate with tape.

8. Replace plate holder in the base with waste tray. Place the top plate squarely over base, making sure that the express prep plate is seated securely. Apply vacuum.

   The flow through is collected in the waste tray.

9. Switch off vacuum. Wash express prep plate by adding 900µl buffer W to each well and applying vacuum. Repeat wash step once more.

10. After buffer W has been drawn through all wells, apply maximum vacuum for an additional 10 minutes to dry the membrane.

    Important: Apply maximum vacuum to dry the membrane. Turn off vacuum regulator or leakage valves if they are used. This step is necessary to remove residual wash buffer. Residual buffer may inhibit subsequent down stream applications.

11. Switch off vacuum, and ventilate the **mdi** VM 96 slowly lift the top plate from the base (do not lift the express prep plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the express prep plate with clean absorbent paper. **Proceed either to step 12 or 13 as desired**.

12. **For elution into provided collection microtubes:**

    Replace waste tray with the collection microtube rack containing collection microtubes. Place the top plate back on the base, making sure that the express prep plate is seated securely.

13. **For elution into a 96-well microplate (not provided):**

    Replace waste tray with an empty collection microtube rack (provided with the **mdi** VM 96). Place a 96-well microplate directly on the rack. Place the top plate back on the base, making sure that the express prep plate is placed securely.

14. To elute DNA, add 100µl of buffer E to the center of each well of the express prep plate, let stand for 1 minute and apply maximum vacuum for 5 minutes, switch off vacuum and ventilate **mdi** VM 96 slowly.

    For increased DNA concentration, an elution volume of 75µl can be used.

18. 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 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. Apply maximum vacuum for 10 minutes to remove the solvent completely.

19. 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.

20. 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 quality 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.

21. Ordering Information

To order please specify as below:

Type		XX	XX	XX	X	Pack Size	
Type	Code					Pack Size	Code
EMPK	EMPK					1	0001

Example:

EMPK	XX	XX	XX	X	0001

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