Genomic DNA Miniprep Kit

>> Animal (Mammalian) Tissue

>> Bacterial Cells

>> Blood

>> Cultured Cells

User Guide

mdi
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Table of Contents

		Page
1.	Introduction	4
2.	Applications	4
3.	Storage Conditions	4
4.	Quality Assurance	5
5.	Safety Information	5
6.	Lot Release Criteria	5
7.	Technical Support	5
8.	Kit contents	6
9.	Specifications	6
10.	How to Begin	7-9
11.	Procedure	10
12.	gDNA Isolation from Animal Tissue	11
	12.1 Principle	11
	12.2 Important Points to be Considered	12
	12.3 Protocol	13-14
13	gDNA Isolation from Bacteria	15
	13.1 Principle	15
	13.2 Important Points to be Considered	16

13.3 Protocol: Gram Negative Bacteria 17-18

13.4 Protocol: Gram Positive Bacteria 19-20

14 gDNA Isolation from Blood 21

14.1 Principle 21

14.2 Important Points to be Considered 22

14.3 Protocol 23-24

15 gDNA Isolation from Cultured Cells 25

15.1 Principle 25

15.2 Important Points to be Considered 26

15.3 Protocol 27-28

16. Trouble Shooting Guide 29-31

17. Product Use Limitations 31

18. Product Warranty and Satisfaction Guarantee 31

19. Ordering Information 32

1. Introduction

The mdi Genomic DNA Miniprep Kit is a fast, economical and easy isolation method of high purity gDNA from animal (mammalian) tissues, blood, bacterial cells and cultured cells. The buffer system provided in the kit allows cell lysis followed by selective binding of gDNA to the spin column.

Purified gDNA is eluted in low-salt buffer or water for variety of downstream applications. This technology does away with the cumbersome methodologies of phenol extraction (associated with slurries formation) as well as ethanol precipitation (associated with anion exchange based purification system) for desalting.

2. Applications

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

3. Storage Conditions

Optimum storage conditions at which all components of the kit can be preserved without alteration in it's quality and performance.

One Year	RT
Longer Storage	2-8°C

Important

In case of any precipitation observed in the buffers, re-dissolve by warming to 56°C for sometime and cool it down to room temperature.

4. Quality Assurance

The mdi Genomic DNA 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 Genomic DNA Miniprep Kit.

6. Lot Release Criteria

Each lot of Genomic DNA 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 systems 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
Collection Tubes	50	250	1000	RT
Buffer EB	15ml	75ml	300ml	RT
Buffer MTL	12ml	60ml	240ml	RT
Buffer LB	12ml	60ml	240ml	RT
Buffer LW1	30ml	150ml	600ml	RT
Buffer LW2	30ml	150ml	600ml	RT
Buffer LE	22ml	110ml	440ml	RT
Proteinase K	1.25ml	6ml	24ml	2-8°C
Hand Book	1	1	1	–
Certificate of Quality	1	1	1	–

Note:

1. Proteinase K is provided in the kit. 1ml of Proteinase K(20mg/ml or 600mAU/ml) is required for 50 spin columns. (In case of Bacterial gDNA isolation the required Proteinase K is 1.25ml).
2. RNase A is not provided in the kit. 200µl of RNase A (100mg/ml or 7 units/µl) is required for 50 spin columns.
3, Lysozyme is not provided. 200mg lysozyme is required for 50 spin columns(only in case of Gram Positive Bacterial gDNA isolation).

9. Specifications

Maximum amount of Tissue	25mg
Maximum Bacterial Cells	2 x 10^9 cells
Maximum Volume of Blood Sample	200µl
Maximum amount of Cultured Cells	$5x10^6$
Capacity of column reservoir	700µl
Binding capacity of membrane (ds DNA)	50µg
Elution volume	200µl

10. How to Begin

A. Sample collection and storage

Do's

1. For obtaining best results, the starting material should be either fresh or that has been immediately frozen and stored at -20 °C or -70 °C.
2. For longer storage, tissue samples can be stored in buffer 'MTL' for six months at ambient temperature after Proteinase K digestion, without showing any reduction in DNA quality.
3. For avoiding accumulation of large amounts of metabolites or formation of very dense cell walls by certain bacterial cultures, it is desirable to harvest cells in the early log phase of growth. Fresh or frozen cell pellets can be used in the procedure.

Don'ts

1. Do not subject the stored samples to repeated freezing and thawing as it leads to reduced DNA size
2. Poor-quality starting material leads to reduced length and yield of purified DNA.

B. Maximum Amount of Starting Material

Do's

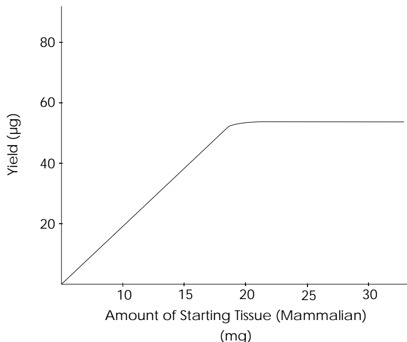
1. For tissues with high cell density and very high DNA contents, reduce the recommended amount of sample.
2. If the DNA content is not known, start with half of the amount specified in the table below and increase gradually to get optimum yields.

Don'ts

Overloading of mdi spin columns lead to significantly lower yields than expected.

Maximum Amount of Starting Material

Sample	Amount
Animal tissue	25 mg
Mammalian blood	200µl
Mouse tail	0.6-1.2 cm
Rat tail	0.6 cm
Bacteria	2 x 10^9 cells
Cultured Cells	5 x 10^6 cells



C. Quantification of Starting Material

Weighing tissues or counting cells is the most efficient way to quantify starting material. However, the approximate guidelines can also be followed.

Animal Tissue

A 2 mm cube (approximately $8mm^3$) of most animal tissue weighs approximately 10-15 mg.

Bacteria

O.D of a bacterial culture measured using a calibrated U.V spectrophotometer gives an idea of the number of cells/ml. However, it is very difficult to give precise and credible recommendations for the correlation.

D. Copurification of RNA

mdi gDNA Miniprep Kit copurifies RNA when present in sample e.g. transcriptionally active tissues like kidney and liver. RNA inhibits some downstream enzymatic reactions.

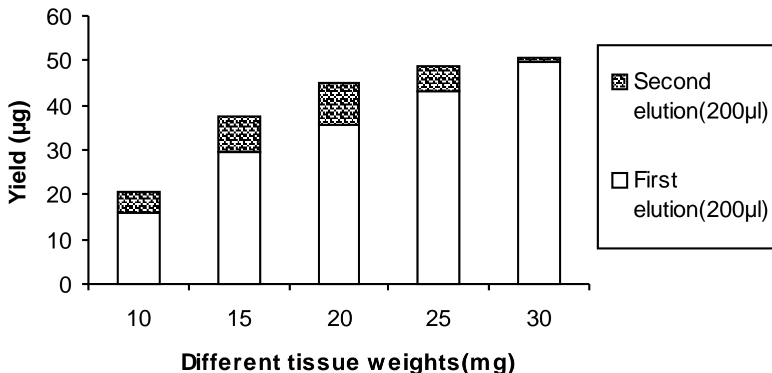
In order to get RNA free gDNA:

1. RNase A should be added to the sample before addition of buffer LB for RNA digestion
2. mdi gDNA Miniprep Kit recommends the use of RNase A stock solution(100mg/ml)
3. RNase amounts are to be adjusted with less concentrated stock solutions but its amount should not exceed 20 µl.

E. Elution

With mdi gDNA kit, highly concentrated gDNA is obtained with single elution step of 200µl.

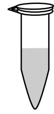
However, in the case of animal tissues with weight ≤20 mg, a second elution step of 200µl is recommended to maximize yields.



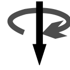
NOTE - Use only buffer LE for elution as elution in water may result in acid hydrolysis of DNA over long term storage.

11. mdi gDNA Miniprep Procedure

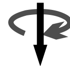
Sample



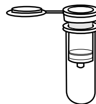
Lyse



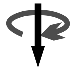
Bind

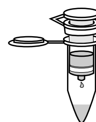


Wash 2X

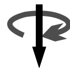


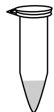
Dry with open lid





Elute





Ultra Pure gDNA

12. gDNA Isolation from Animal (Mammalian) Tissue

12.1 Principle

Obtaining highly pure gDNA from animal tissue using mdi gDNA Miniprep Kit involves:

Lysis and splicing of the animal tissue

To efficiently lyse the animal tissue, cut it into small pieces before addition of buffer MTL and Proteinase K. To minimize lysis time (to 20 minutes) grind the sample in liquid nitrogen before addition of buffer MTL and Proteinase K.

For RNA free DNA yield, add RNase A for complete digestion of RNA.

Capturing of gDNA on Spin Column

In order to facilitate adsorption of gDNA onto the spin column, suitable conditions of salt concentration and pH are required, which is achieved by addition of binding buffer LB.

Washing

Subsequent to DNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away. Washing is done by buffer LW1 and LW2.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency. Elution occurs at basic conditions and low salt concentration. Elution is done with buffer LE.

Yield

Yield of gDNA depend on amount and quality of starting material.

Type of Tissue	Amount	DNA Yield (µg)
Liver	25 mg	24.4
Kidney	25 mg	30.2
Lung	25 mg	10
Spleen	10 mg	15.4
Mouse Tail	25 mg	15

12.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum weight of sample tissue should be 25mg.
3. The optimum length of mouse tail should be 0.4-0.6cm.

Centrifugation

1. All centrifugation steps should be carried out at room temperature at $\geq$ 10,000 rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. For efficient lysis cut tissue into small pieces. To reduce lysis time grind the sample in liquid nitrogen before addition of buffer MTL and Proteinase K.
2. Add RNase A to sample before addition of buffer LB for complete removal of RNA.
3. Proteinase K should be added to sample after addition of buffer MTL to inactivate nucleases that can degrade DNA during purification.
4. In case of any precipitation in LB and MTL buffers, re-dissolve by warming to 56°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with open lid for 1 minute at $\geq$ 10,000 rpm.
2. Flow through should be properly discarded before centrifugation otherwise residual buffer will not be removed and may inhibit enzymatic reactions.

Elution

1. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 2-3 minutes.
2. For obtaining highly concentrated gDNA, elution should be done in two successive steps with buffer LE in separate micro centrifuge tubes.

12.3 Protocol

1. Cut up to 25mg tissue (upto 10mg for spleen tissue) into small pieces, and place in a 1.5 ml microcentrifuge tube. In case of rodent tail, cut one (rat) or two (mouse) 0.4-0.6 cm lengths and place them into a 1.5ml microcentrifuge tube.

   Note: Rodent tail should not exceed 1.2cm (mouse) or 0.6cm (rat). It is recommended to use 0.4-0.6cm tail length of adult rodent.

2. Add 180µl buffer MTL and 20µl of proteinase K from stock (20mg/ml or 600mAU/ml). Mix throughly by vortexing and incubate at 56°C until complete tissue lysis is achieved. Vortex occasionally or place in a thermo mixture, shaking water bath or on a rocking platform to disperse the sample. Lysis is usually complete in 1-3 hrs. For rodent tails, it takes 6-8 hrs. Samples can be lysed overnight without affecting DNA yield.

   Note: To reduce lysis time, grind the sample in liquid nitrogen before addition of buffer MTL and proteinase K.

   If residual tissue remains undigested in case of rodent tails then centrifuge lysate for 5 minutes at 10,000 rpm, transfer supernatant to a fresh microcentrifuge tube. Proceed to the next step.

3. For RNA free DNA, add 4µl of RNase A from 100mg/ml stock (not provided), mix by vortexing and incubate at RT for 2 minutes.

4. Add 200µl Buffer LB to the sample, mix throughly by vortexing. Then add 200µl Ethanol and mix again throughly by vortexing.

5. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥10,000 rpm for 1 min. Discard the flow through

   Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample, balance and spin at ≥ 10,000 rpm for 1min. Discard the flow through.

If the sample does not pass after 1 min then centrifuge for an additional 1 minute.

6. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW1 by centrifuging for 1 minute at ≥10,000 rpm. Discard the flow through.

7. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW2 by centrifuging for 1 minute at ≥10,000 rpm. Discard the flow through.

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

   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 microcentrifuge tube (not provided). Elute the bound gDNA by adding 200µl of buffer LE directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥10,000 rpm for 1 minute.

   Note: Repeat elution step once more to have maximum DNA yield in a new microcentrifuge tube.

13. gDNA Isolation from Bacteria

13.1 Principle

Obtaining highly pure gDNA from bacterial cells using mdi gDNA Miniprep Kit involves:

Lysis of bacterial cells

For efficient lysis resuspend bacterial pellet completely and ensure cell wall lysis at 37°C and Proteinase K incubation at 56°C.

Capturing of gDNA on Spin Column

In order to facilitate adsorption of gDNA onto the spin column, suitable conditions of salt concentration and pH are required, which is achieved by addition of binding buffer LB.

Washing

Subsequent to DNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away. Washing is performed by buffer LW1 and LW2.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency. Elution occurs at basic conditions and low salt concentration. Elution is performed with buffer LE.

Yield

Yield of gDNA depend on amount and quality of starting material.

Typical gDNA yields from Bacterial Cells

Sample	O.D	Cell Count	DNA Elution (Ist)	DNA Elution (IInd)	Total DNA (µg)
DH_5a	3.0	App. $2x10^9$	14	8	22
XL-1 Blue	3.9	App. $2x10^9$	14.2	3.6	17.8
Bacillus subtilis	3.0	App. $2x10^9$	15.6	7	22.6

13.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum number of cells should be $2x10^9$.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature at $\geq$ 10,000 rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. For efficient lysis resuspend bacterial pellet completely. Ensure cell wall lysis at 37°C and Proteinase K incubation at 56°C.
2. Add RNase A to the sample before addition of buffer LB for complete removal of RNA.
3. Proteinase K should be added to sample before addition of buffer LB to inactivate nucleases that can degrade DNA during purification.
4. In case of any precipitation in LB and MTL buffers, re-dissolve by warming to 56°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with open lid for 1 minute at $\geq$ 10,000 rpm.
2. Flowthrough should be properly discarded before centrifugation otherwise residual buffer will not be removed and may inhibit enzymatic reactions.

Elution

1. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 2-3 minutes.
2. For obtaining highly concentrated gDNA, elution should be done in two successive steps with buffer LE in separate micro centrifuge tubes.

13.3 Protocol: Isolation of gDNA from Gram Negative Bacteria

This protocol has been developed for purification of total gDNA from Gram negative bacteria such as E. Coli.

Note:

1. Heat a water bath to 56°C.
2. Arrange ethanol (96-100 %).

Procedure

1. Centrifuge bacterial culture (Max. $2x10^9$ cells) in a microcentrifuge tube at 10,000 rpm for 5 minutes. Remove all the traces of supernatant by inverting the open microcentrifuge tube until all medium has been drained.
2. Resuspend the pellet in 180µl of Buffer MTL completely by pipetting up and down.
3. Add 20 µl of Proteinase K from stock 20mg/ml or 600mAU/ml. Mix throughly by vortexing and incubate at 56°C for 30 minutes. Vortex 2-3 times during incubation to disperse the sample.
4. Add 4µl of RNaseA from 100mg/ml stock or 28 units, mix by vortexing and incubate at RT for 2 minutes.
5. Add 200µl Buffer LB to the sample, mix throughly by vortexing. Then add 200µl Ethanol and mix again throughly by vortexing.
6. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥10,000 rpm for 1 min. Discard the flow through.

   Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample, balance and spin at ≥ 10,000 rpm for 1min. Discard the flow through.

   If the sample does not pass after 1 min then centrifuge for an additional 1 minute.

7. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW1 by centrifuging for 1 minute at ≥10,000 rpm. Discard the flow through.
8. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW2 by centrifuging for 1 minute at ≥10,000 rpm. Discard the flow through.
9. Place the spin column with open lid in the same collection tube and centrifuge for 1 minute at ≥10,000 rpm.

   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.
10. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided). Elute the bound gDNA by adding 200µl of buffer LE directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥10,000 rpm for 1minute.

    Note: Repeat elution step once more to have maximum DNA yield in a separate microcentrifuge tube.

13.4 Protocol: Isolation of gDNA from Gram Positive Bacteria

This protocol has been developed for purification of total gDNA from Gram positive bacteria such as Bacillus subtilis.

Note:

1. Prepare lysozyme stock 20mg/ml in buffer EB and store at -20°C.

2. Heat a water bath to 37°C.

3. Arrange ethanol (96-100 %).

Procedure

1. Centrifuge bacterial culture(Max. $2x10^9$ cells) in a microcentrifuge tube at 10,000 rpm for 5 minutes. Remove all the traces of supernatant by inverting the open microcentrifuge tube until all medium has been drained.

2. Resuspend the pellet in 180µl of Lysozyme (20mg/ml stock in Buffer EB) completely by pipetting up and down.

3. Incubate at 37°C for 30 minutes and mix 2-3 times during incubation.

4. Add 4µl of RNaseA from 100mg/ml stock or 28 units (not provided), mix by vortexing and incubate at RT for 2 minutes.

5. Add 25 µl of Proteinase K from stock 20mg/ml or 600mAU/ml. Mix throughly by vortexing then add 200µl of buffer LB, mix again throughly by vortexing and incubate at 56°C for 30 minutes. Vortex 2-3 times during incubation to disperse the sample.

6. Add 200µl of Ethanol (96-100%) and mix throughly by vortexing.

7. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥10,000 rpm for 1min. Discard the flow through.

   Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample, balance and spin at ≥ 10,000 rpm for 1min. Discard the flow through.

If the sample does not pass after 1 min then centrifuge for an additional 1 minute.

8. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW1 by centrifuging for 1 minute at $\geq$10,000 rpm. Discard the flow through.

9. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW2 by centrifuging for 1 minute at $\geq$10,000 rpm. Discard the flow through.

10. Place the spin column with open lid in the same collection tube and centrifuge for 1 minute at $\geq$10,000 rpm.

    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.

11. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided).

    Elute the bound gDNA by adding 200µl of buffer LE directly to the center of the column membrane and let it stand for 1 minute. Spin at $\geq$10,000 rpm for 1 minute.

    Note: Repeat elution step once more to have maximum DNA yield in a separate microcentrifuge tube.

14. gDNA Isolation from Blood

14.1 Principle

Obtaining highly pure gDNA from blood using mdi gDNA Miniprep Kit involves:

Lysis of Blood Sample

For efficient lysis mix blood sample completely with Proteinase K and buffer LB, then incubate at 56°C.

Capturing of gDNA on Spin Column

In order to facilitate adsorption of gDNA onto the spin column, optimum conditions of salt concentration and pH are required, which is achieved by addition of binding buffer LB.

Washing

Subsequent to gDNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away.

Washing is done by buffer LW1 and LW2.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency. Elution occurs at basic conditions and low salt concentration. Elution is performed with buffer 'LE'.

14.2 Important Points to be Considered

Starting Material

1. Fresh samples should be used.
2. Maximum amount of blood sample should be 200µl.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature at ≥ 10,000 rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. For efficient lysis, mix blood sample completely with Proteinase K and buffer LB, then incubate at 56°C.
2. Add RNaseA to the sample before addition of buffer LB for complete removal of RNA.
3. Proteinase K should be added to sample before addition of buffer LB to inactivate nuclease that can degrade DNA during purification.
4. In case of any precipitation in buffer LB, re-dissolve by warming to 56°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with open lid for 1 minute at ≥ 10,000 rpm.
2. Flowthrough should be properly discarded before centrifugation otherwise residual buffer will not be removed and may inhibit enzymatic reactions.

Elution

1. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 2-3 minutes.
2. For obtaining highly concentrated gDNA, elution should be done in two successive steps with buffer LE in separate micro centrifuge tubes.

14.3 Protocol

Note: Things to do before starting

1. Heat a water bath at 56°C
2. Arrange ethanol (96-100%)

Procedure:

1. Pipet 20µl Proteinase K into the bottom of a 1.5ml microcentrifuge tube.
2. Add 200µl blood sample to the microcentrifuge tube and mix well by pipetting up and down.

   Note: It is possible to add Proteinase K to the sample that have already been dispensed into microcentrifuge tube.
3. Add 4µl of RNase A from 100mg/ml stock or 28 units and mix well by pipeting up and down.
4. Add 200µl buffer LB to the sample, mix throughly by vortexing.

   Note: Do not add Proteinase K directly to buffer 'LB'.
5. Incubate at 56°C in water bath for 10 minutes. Mix 1-2 times by inverting the tube during incubation.
6. Briefly centrifuge the sample after incubation to remove drops from inside of lid.
7. Add 200µl Ethanol, mix well by vortexing and briefly centrifuge the sample to remove drops from inside of lid.
8. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at $\geq$ 10,000 rpm for 1 minute. Discard the flow through.

   Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample balance and spin at $\geq$ 10,000 rpm for 1 minute. Discard the flow through. If the sample does not pass after 1 minute then centrifuge for additional 1 minute.

9. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW1 by centrifuging for 1 minute at ≥ 10,000 rpm. Discard the flowthrough.
10. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW2 by centrifuging for 1 minutes at ≥ 10,000 rpm. Discard the flowthrough.
11. Place the spin column with open lid in the same collection tube and centrifuge for 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.
12. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)

    Elute the bound gDNA by adding 200µl of buffer LE directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥ 10,000rpm for 1 minute.

    Note: Repeat elution step once more to have maximum DNA yield in a separate microcentrifuge tube.

15. gDNA Isolation from Cultured Cells

15.1 Principle

Obtaining highly pure gDNA from cultured cells using mdi gDNA Miniprep Kit involves:

Lysis and Resuspension of Cultured Cells

For efficient lysis, the pellet should be completely mixed with buffer PBS and Proteinase K, then incubate at 56°C after adding buffer LB.

Capturing of gDNA on Spin Column

In order to facilitate adsorption of gDNA onto the spin column, optimum conditions of salt concentration and pH are required, which is achieved by addition of binding buffer LB.

Washing

Subsequent to gDNA binding, unwanted components like proteins, carbohydrates and cell debris are washed away.

Washing is done by buffer LW1 and LW2.

Elution

Salt concentration and pH of elution buffer is important for elution efficiency. Elution occurs at basic conditions and low salt concentration. Elution is performed with buffer 'LE'.

15.2 Important Points to be Considered

Starting Material

1. Freshly prepared PBS buffer should be used.
2. Maximum number of cultured cells should be $5x10^6$.
3. It is recommended to use less than the maximum number of cells with cell lines having high degree of ploidy.

Centrifugation:

1. All centrifugation steps should be carried out at room temperature at $\geq$ 10,000 rpm.
2. In case of choking of spin column, increase centrifugation time.

Lysis

1. For efficient lysis, mix the pellet completely with Proteinase K and buffer PBS, then incubate at 56°C after adding buffer LB.
2. Add RNaseA to the sample before addition of buffer LB for complete removal of RNA.
3. Proteinase K should be added to sample before addition of buffer LB to inactivate nucleases that can degrade DNA during purification.
4. In case of any precipitation in buffer LB, re-dissolve by warming to 56°C for few minutes.

Washing

1. To remove residual wash buffer, spin the column with open lid for 1 minute at $\geq$ 10,000 rpm.
2. Flow through should be properly discarded before centrifugation otherwise residual buffer will not be removed and may inhibit enzymatic reactions.

Elution

1. Elution buffer must be dispensed on to the center of column. For maximum elution efficiency, incubation time should be increased by 2-3 minutes.
2. For obtaining highly concentrated gDNA, elution should be done in two successive steps with buffer LE in separate micro centrifuge tubes.

15.3 Protocol

The protocol has been developed for purification of total gDNA from cultured cells

Note:

1. Heat a water bath to 56 C.
2. Arrange ethanol (96-100%).
3. Prepare PBS, pH 7.2 (50 mM potassium phosphate , 150 mM NaCl).

Procedure

1. Centrifuge cultured cells (maximum 5 x 10^6) at 8,000 rpm for 5 minutes. Discard the supernatant carefully.
2. Resuspend the pellet in 200µl of buffer PBS, add 20 µl of Proteinase k and mix thoroughly by vortexing.

   Note: When using frozen cell pellet ,allow cells to thaw before adding PBS until the pellet can be dislodged by gently flicking the tube.
3. Add 4µl of RNase from 100 mg/ml stock or 28 units and mix well by pipetting up and down.
4. Add 200µl buffer LB to the sample, mix thoroughly by vortexing .

   Note: Do not add Proteinase K directly to buffer ' LB'.
5. Incubate at 56 °C in water bath for 10 minutes. Mix 1-2 times by inverting the tube during incubation.
6. Briefly centrifuge the sample after incubation to remove drops from inside of lid.
7. Add 200µl Ethanol, mix well by vortexing and briefly centrifuge the sample to remove drops from inside of lid.

8. Place the spin column in the collection tube, pass the above sample through the spin column by spinning at ≥ 10,000 rpm for 1 minute. Discard the flow through.

   Note: Maximum volume of the column reservoir is 700µl. For sample volumes >700µl, simply load remaining sample balance and spin at ≥ 10,000 rpm for 1 minute. Discard the flow through. If the sample does not pass after 1 minute then centrifuge for additional 1 minute.

9. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW1 by centrifuging for 1 minute at ≥ 10,000rpm. Discard the flowthrough.

10. Place the spin column in the same collection tube. Wash the column with 500µl of buffer LW2 by centrifuging for 1 minute at ≥ 10,000rpm. Discard the flowthrough.

11. Place the spin column with open lid in the same collection tube and centrifuge for 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.

12. Place the spin column in a fresh 1.5ml microcentrifuge tube (not provided)

    Elute the bound gDNA by adding 200µl of buffer LE directly to the center of the column membrane and let it stand for 1 minute. Spin at ≥ 10,000rpm for 1 minute.

    Note: Elute with 100µl to increase the gDNA concentration but overall gDNA will be decreased.

    Repeat elution step once more to have maximum DNA yield in a separate microcentrifuge tube.

    Do not elute more then 200µl into a 1.5ml micro centrifuge tube because the spin column will come into contact with the eluate.

16. Trouble Shooting Guide

A. Little or no Yield of gDNA

Problem	Solution
1. Overloading of spin column for:	a. Animal Tissue should not weigh more than 25mg. b. Bacterial Cells: Ensure optimum cell density through optimum growth conditions. c. Blood Cells: Can happen due to very high cell (Leukocyte)density, as in case of infected donors. d. Cultured Cells: Can happen due to high cell density.
2. Precipitates in buffer	In case of any precipitates in buffers, re- dissolve by warming to 56°C before use.
3. Spin column choked	Increase centrifugation time while passing sample through the spin column.
4. Insufficient Lysis	Ensure incubation temperature of 56 °C. a. Animal Tissue: Cut tissue sample into small pieces to ensure efficient lysis. For hard tissue like rodent tail ensure a minimum incubation time of 6-8 hours. Increase incubation time and vortex occasionally during incubation. b. Bacterial Cells: Ensure addition of lysozyme and Proteinase K to the sample. Increase incubation time till complete lysis is achieved. c. Blood / Cultured Cells: Check that Proteinase K was added to the sample.

5. Improper dispensing of elution buffer	The elution buffer must be dispensed properly on to the centre of the column membrane.
6. Insufficient incubation of elution buffer in the column membrane	Increase incubation time by 2-3 minutes.
7. Insufficient mixing of sample with buffer LB and ethanol before loading on to the spin column	Add buffer LB to the sample first and mix by vortexing. Then add ethanol to the sample and again mix by vortexing.
8. Poor Quality Sample	Use fresh sample or sample stored at -20°C to -70°C. Avoid repeated freezing and thawing of sample.
9. Improper storage of blood samples	For short term storage blood samples should be stored at 4°C in tubes containing a standard anticoagulant (E.D.T.A.). For long term, store at-70°C with anticoagulant.
B: Low quality DNA	
1. Nuclease contamination	Use autoclaved plastic and glassware.
2. RNA contamination	RNase digestion is insufficient. Check that RNase A is added to the lysate.
3. Sheared Genomic DNA	Avoid vigorous mixing.
C: DNA does not perform well	
Residual wash buffer in eluate	Spin the column with open lid for 2-3 minutes extra at $\geq$10,000 rpm, to remove residual wash buffer completely.
D: Low A_{260}/A_{280} ratio	
DNA diluted with water instead of buffer	Use buffer LE to dilute the sample before measuring A_{260}/A_{280} ratio for purity.

E: High A_{260}/A_{280} ratio

High level of residual RNA	RNase A treatment should be performed as per the protocol.

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	P	Pack Size

Type	Code
CTGK	CTGK

Pack Size	Code
50	0050
250	0250
1000	1000

Example:

CTGK	XX	XX	XX	P	0250

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