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Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) — Product Insert

Product # 62500

Norgen's Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) provides a fast and reproducible high-throughput method for isolating genomic DNA from cultured cells as well as various tissue samples, bodily fluids and nasal or throat swabs. The purified DNA is of the highest quality, and is compatible with a number of downstream research applications including PCR, NGS and microarray analysis. Using this kit DNA can be extracted either manually or by using automation platform IsoPure™. The protocol can also be easily modified by making minor changes to be able to run on other magnetic bead-based automation platforms such as KingFisher™ Flex 96 and Hamilton MagEx STAR platforms.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that bind DNA under optimized binding conditions. Norgen's Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) allows for the isolation of genomic DNA from various types of animal tissues or cell samples. Different sample lysate preparation steps are applied, including a Proteinase K treatment. Next, Magnetic Beads A and ethanol are added to the clean supernatant, and the resulting solution is subjected to magnetic force to separate the magnetic beads from the solution. Only the DNA will bind to the magnetic beads, while most of the proteins will be removed in the supernatant. The bound DNA is then washed with Solution WN and 70% ethanol in order to remove any remaining impurities and the purified total DNA is eluted with the Elution Buffer B. The purified DNA can be used in a number of downstream applications.

Specifications

Kit Specifications	
Number of Preps	192
Maximum Cells and Tissue Input	20 mg of animal tissue 3 x 10^6 cells
Average Yield *	8-10 µg (20 mg of animal tissue) 8-12 µg (3 x 10^6 cells)
Average Purity (OD260/280)	1.8 – 1.9
Time to Complete 96 Purifications (automated)	15 minutes (hands-on time)
Time to Complete 96 Purifications (manual)	60 minutes (hands-on time)

* Average DNA yield will vary depending on the type of samples

Kit Components

Component	Product #62500 (192 samples)
Lysis Buffer B	1 x 40 mL 1 x 20 mL
Proteinase K in Storage Buffer	4 mL
Solution WN	55 mL
Elution Buffer B	1 x 30 mL 1 x 15 mL
Magnetic Beads A	8.5 mL
96-Well Plate (Manual Extraction)	2
96-Well Elution Plate (Manual Extraction)	2
Adhesive Tape	2
Product Insert	1

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 1 year after the date of shipment. The kit contains a ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 1 year after the date of shipment when stored at room temperature.

Advantages
- Isolate genomic DNA from cultured cells as well as various tissue types
- Fast, reproducible and easy processing using a Magnetic Bead System
- Isolate high quality genomic DNA
- High throughput and compatible with automation robotic system
- Recovered genomic DNA is compatible with various downstream applications

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use.
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

Solution WN contains guanidinium salts and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Cells and tissue of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.

Customer-Supplied Reagents and Equipment
- Magnetic Bead 96-Well Separation Plate (For manual Isolation)
- Adhesive Tape (For manual Isolation) – We recommended Norgen Cat. 28394 Adhesive Tape – 100 Units
- IsoPure™ 96 or IsoPure™ Mini Purification System (For automated Isolation)
- Multi-channel micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- 96 – 100% ethanol
- Temperature adjustable (37°C, 55°C, 65ºC) incubator(s)
- Nuclease-free water
- Phosphate Buffered Saline (PBS) – (for Cultured Animal Cells protocol)
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer - (for Animal Tissue protocol)
- Syringe with a 22G needle - (for Animal Tissue protocol)
- Orbital Shaker
- RNase A (optional)

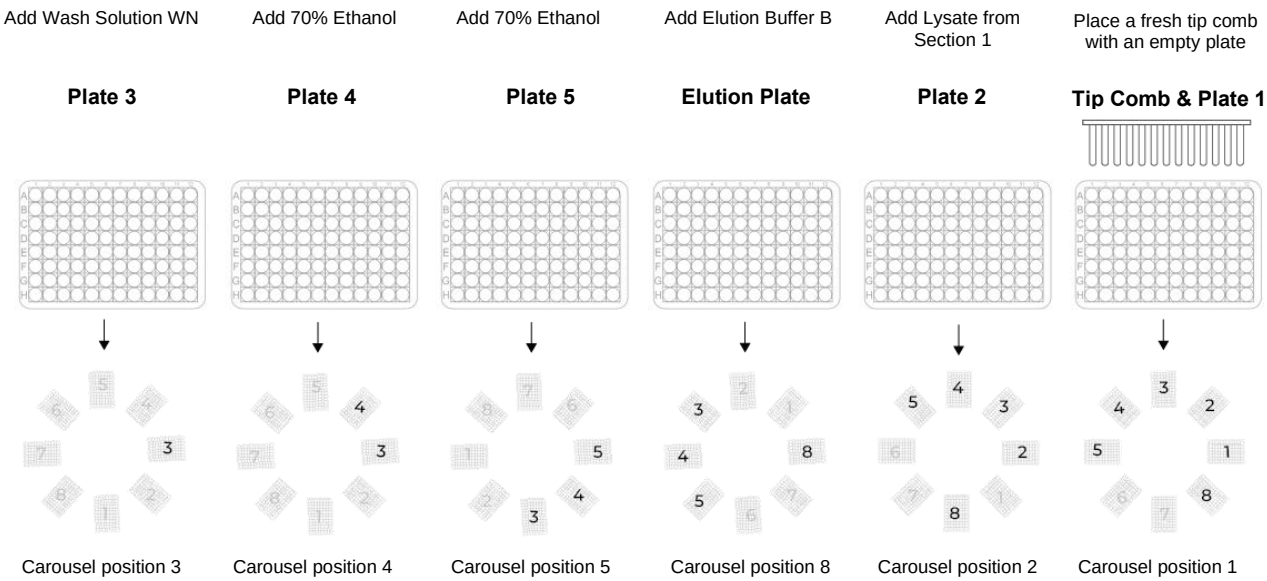
Procedure

Notes prior to use:
- The procedure provides information and steps to follow to extract DNA using Automated as well as Manual method.
- In each method, the steps for preparing the lysate are different depending on the starting material **(Section 1)**. However, the subsequent steps are the same in all cases **(Section 2)**.
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- **Always** vortex the **Magnetic Beads A** before use.
- **Always** vortex the **Proteinase K** before use.

- Preheat the incubator(s) according to the temperatures required (37°C or 55°C or 65ºC). For automation procedures, the automation platform that is being used may have a heat block to provide appropriate temperatures for incubation and thus an incubator might not be required. IsoPure™ instruments come with heat blocks and the scripts provided includes the incubation at required temperature.
- Prepare a working concentration of the **Solution WN** by adding 73 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 128mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Flow Chart

Procedure for using Norgen's Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) –IsoPure™ 96



Run Instrument

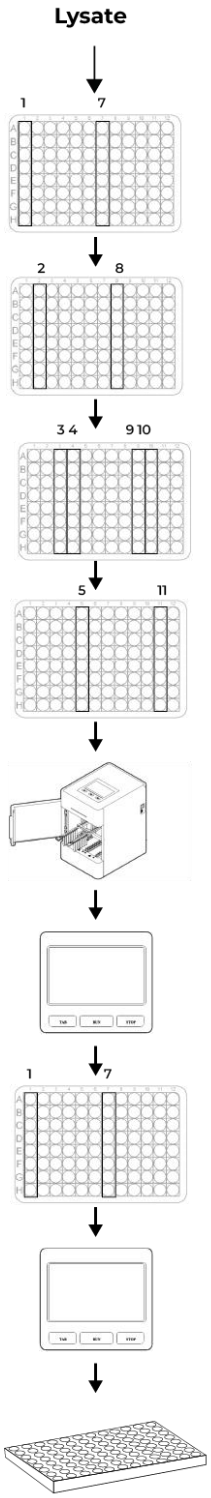
Add ethanol & bead mix to Plate 2 when prompted

Press "OK" after placing plate 2 back at position 2

Run Complete. Take Elution plate and store DNA.

Flow Chart

Procedure for using Norgen's Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) –IsoPure™ Mini



Add Lysate

Add Solution WN

Add 70% Ethanol

Add Elution Buffer B

Install fresh Tip Comb

Run Program "CNTDNA16"

Add Ethanol and Bead Mix

Press "OK"

Transfer DNA to fresh Plate

I. Genomic DNA Isolation using Automation Platforms

Procedure for IsoPure™ Mini or IsoPure™ 96 Automation System

The procedure outlined below is for extraction of DNA using automation platforms IsoPure™ 96 (Cat. AP1096) and IsoPure™ Mini (Cat. AP1016). This procedure can easily be adapted to other automation platforms such as KingFisher™ Flex 96 and Hamilton MagEx STAR platforms.

Set-up the IsoPure™ Plates

Notes Prior to Use

- Only use plates that are compatible with the instrument that is being used. Use 96 deep well plates for IsoPure 96 (Cat. AP1096-DWP), 96 well elution plates for IsoPure™ (Cat. AP1096-ELP) and Tip Comb for IsoPure 96 (Cat. AP1096-TC) while working with IsoPure™ 96 Instruments. Use 96 deep well plates for IsoPure 96 (Cat. AP1096-DWP) and 8-place magnetic tip comb for IsoPure Mini (Cat. AP1016-MC) while working with IsoPure™ Mini Instruments.
- The numbering of plates is based on where it should be placed on the carousel in the instrument. Use the carousel rotation keys on the instrument or select the carousel position on the screen to bring it to the right position as mentioned in the flow chart.
- IsoPure™ 96 uses multiple plates for DNA extraction while IsoPure™ Mini uses single plate for DNA extraction.
- For IsoPure™ 96 most of the lysate preparation is performed in plate 2 while other plates are used for performing washing and elution steps.
- For IsoPure™ Mini the lysate preparation is performed in column 1 and/or 7 while washing and elutions steps could be performed in other columns of the same plate.
- For IsoPure™ 96, DNA elution step is always performed in plate 8.
- Prepare the plates and label them based on the sample type as mentioned in the tables below.

Table 1. Set-up for Animal Tissue DNA Extraction

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2.**

Component	IsoPure™ Mini	IsoPure™ 96	Location on Carousel	Amount
Tip Comb	In the instrument	Plate 1	1	Empty Plate
Lysate	Column 1 / Column 7	Plate 2	2	Variable
Wash WN	Column 2 / Column 8	Plate 3	3	500 µL
70% Ethanol	Column 3 / Column 9 and Column 4 / Column 10	Plate 4 & Plate 5	4 & 5	500 µL
Elution Buffer B	Column 5 / Column 11	Elution Plate	8	50-100 µL

Table 2. Set-up for Cells and Viral Suspension DNA Extraction

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2.**

Component	IsoPure™ Mini	IsoPure™ 96	Location on Carousel	Amount
Tip Comb	In the instrument	Plate 1	1	Empty Plate
Sample	Column 1 / Column 7	Plate 2	2	Variable
Proteinase K	Column 1 / Column 7	Plate 2	2	20 µL
Lysis Buffer B	Column 1 / Column 7	Plate 2	2	300 µL
Wash WN	Column 2 / Column 8	Plate 3	3	500 µL
70% Ethanol	Column 3 / Column 9 and Column 4 / Column 10	Plate 4 & 5	4 & 5	500 µL
Elution Buffer B	Column 5 / Column 11	Elution Plate	8	50-100 µL

Section 1. Lysate Preparation from Various Sample Types

1A. Lysate Preparation from Animal Tissues

Notes Prior to Use

- Fresh or frozen tissues may be used for the procedure.
- Tissues should be immediately frozen and stored at -20°C or -70°C. Tissues may be stored at -70°C for several months.
- It is recommended that no more than 20 mg of tissue be used.

1A. Lysate Preparation from Animal Tissues

a) Excise up to 20 mg of tissue sample. Either frozen or fresh tissue may be used.
b) Homogenize the sample into a fine powder in liquid nitrogen using a mortar and pestle, then transfer into a nuclease-free microfuge tube.
c) Add 300 µL **Lysis Buffer B** to the tissue sample.

Note: The sample can also be homogenized using tools such as a rotor-stator homogenizer or a microfuge-size pestle in the provided Lysis Solution.

d) Use a syringe with a 22G needle to further homogenize the lysate by passing through the needle 10 times.
e) Add 20 µL of **Proteinase K** (vortex before use) to the lysate. Mix well by gentle vortexing.
f) Transfer the entire lysate to **Plate 2** (IsoPure™ 96) or **Column 1/Column 7** (IsoPure™ Mini).
g) Proceed to **Section 2:** DNA Isolation from All Types of Lysates.

1B. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- Cells grown in suspension or monolayer may be used.
- The maximum recommended input of cells is 3 x 10^6. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen cell pellets should not be subjected to repeated cycles of freeze and thaw prior to beginning the protocol.

1B (i). Cell Lysate Preparation from Cells Growing in a Monolayer

a) Add 300 µL **Lysis Buffer B** and 20 µL **Proteinase K** to all the wells in **Plate 2** (IsoPure™ 96) or **Column 1 and/or Column 7** (IsoPure™ Mini) where the sample will be added. For ease of use, prepare a mixture of Lysis Buffer B and Proteinase K. (For example, if DNA is to be extracted from 10 samples, mix 3 mL of **Lysis Buffer B** and 200µL of **Proteinase K**). Make 10% extra volume to avoid volume loss due to pipetting.

b) Detach cells by standard trypsinization method or cell scraper. Transfer an appropriate amount to a 1.5 mL microfuge tube (not provided). The maximum recommended input of cells is 3 x 10^6.
c) Collect cells by centrifugation at no more than 200 x g (~2,000 RPM) for 10 minutes. Discard the supernatant.
d) Add 100 µL of phosphate buffered saline (PBS) to the cell pellet. Mix and transfer the cells dissolved in PBS to **Plate 2** (IsoPure™ 96) and **Column 1 and/or Column 7** (IsoPure™ Mini).
e) Proceed to **Section 2:** DNA Isolation from All Types of Lysates.

1B (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a) Add 300 µL **Lysis Buffer B** and 20 µL **Proteinase K** to all the wells in **Plate 2** (IsoPure™ 96) and **Column 1 and/or Column 7** (IsoPure™ Mini) where the sample will be added. For ease of use, prepare a mixture of Lysis Buffer B and Proteinase K. (For example, if DNA is to be extracted from 10 samples, mix 3 mL of **Lysis Buffer B** and 200 µL of **Proteinase K**). Make 10% extra volume to avoid volume loss due to pipetting.
b) Transfer an appropriate amount of cells to a 1.5 mL microfuge tube (not provided). The maximum recommended input of cells is 3 x 10^6.
c) Collect cells by centrifugation at no more than 200 x g (~2,000 RPM) for 10 minutes. Discard the supernatant.
d) Add 100 µL of phosphate buffered saline (PBS) to the cell pellet. Mix and transfer the cells dissolved in PBS to **Plate 2** (IsoPure™ 96) or **Column 1 and/or Column 7** (IsoPure™ Mini).
e) Proceed to **Section 2:** DNA Isolation from All Types of Lysates.

1C. Lysate Preparation for Viral DNA

Notes Prior to Use

- For the isolation of integrated viral DNA, follow **Section 1A** if the starting material is animal tissue and follow **Section 1B** if the starting material is cell culture.
- For the isolation of DNA from free viral particles, follow the protocol provided below.
- Up to 150 µL of viral suspension can be processed.
- Fresh samples are recommended. Frozen samples may be used, however the yield of genomic DNA may be decreased.

1C. Lysate Preparation for Viral DNA

a) Add 300 µL **Lysis Buffer B** and 20 µL **Proteinase K** to all the wells in **Plate 2** (IsoPure™ 96) and **Column 1 and/or Column 7** (IsoPure™ Mini) where the sample will be added. For ease of use, prepare a mixture of Lysis Buffer B and Proteinase K. (For example, if DNA is to be extracted from 10 samples, mix 3 mL of Lysis Buffer B and 200 µL of Proteinase K). Make 10% extra volume to avoid volume loss due to pipetting.
b) Transfer up to 150 µL of viral suspension to **Plate 2** (IsoPure™ 96) and **Column 1 and/or Column 7** (IsoPure™ Mini).
c) Proceed to **Section 2:** DNA Isolation from All Types of Lysates.

Section 2: DNA Isolation from All Types of Lysates

Table 3. 96-100% ethanol and Magnetic Bead volume to be added to lysate of each sample type.

Sample Type	Ethanol Volume (µL)	Magnetic Bead Volume (µL)	Total Volume (µL)
Tissue	300	40	340
Cells (Monolayer)	400	40	440
Cells (Suspension)	400	40	440
Viral Suspension	450	40	490

2.1 DNA Isolation using IsoPure™ 96 System

a) Setup all the plates according to **Table 1 or 2** as per the sample being isolated, add 500 µL of **Solution WN** into **Plate 3** and keep it at **position 3**.
b) Add 500 µL freshly prepared **70% ethanol** to **plate 4 & 5** and keep it at **position 4 & 5** respectively.
c) Add 50-100 µL of **Elution Buffer B** to the **elution plate** and place it at **position 8**.
d) Place sample plate **(Plate 2)** in the machine at **position 2**.
e) Place a clean tip comb in an empty plate **(Plate 1)** and keep it at **position 1.**
f) After setting up, run the program **CNTDNA96**.
g) Prepare a mixture of **96-100% ethanol** and **Magnetic Beads A** as per **Table 3**. For ease of pipetting, it is recommended to prepare a bulk mix containing **96-100% ethanol** and **Magnetic Beads A.** We recommend a 10% overage to compensate for pipetting errors. Make sure to vortex the bulk mix before adding it to the lysate. It is recommended to prepare the bulk fresh every time a new DNA isolation is performed.
h) After 1 hour of incubation, the instrument will pause and will allow the user to add mixture of ethanol and magnetic beads prepared in **step g.** Open the cover and remove the plate from the instrument and add the prepared mixture from **step g** to **Plate 2**.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the lysate after addition of **96-100% ethanol** and **Magnetic Beads A** mixture to **Plate 2** during step **g**. If RNase treatment is performed, make sure to use **CNTDNA96R** (IsoPure™ 96) **program instead of CNTDNA96** to include an incubation step of 37°C for 15 minutes.

i) Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.
j) The instrument will prompt after the procedure is complete. Remove the elution plate from the instrument and the DNA is now ready for further downstream processing.
k) The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at –20°C for long-term storage.

2.2 DNA Isolation using Isopure™ Mini System

a. Setup the plates according to **Table 1 or 2** as per the sample being isolated, add 500 µL of **Solution WN** into **Column 2/8**.
b. Add 500 µL freshly prepared **70% ethanol** to **Column 3 and/or Column 9** and **Column 4 and/or Column 10**.
c. Add 50-100 µL of **Elution Buffer B** to **Column 5 and/or 11**.
d. Place a clean tip comb into IsoPure™ Mini.
e. After setting up, place the plate into the instrument and run the program **CNTDNA16**.
f. Prepare a mixture of **96-100% ethanol** and **Magnetic Beads A** as per **Table 3.** For ease of pipetting, it is recommended to prepare a bulk mix containing **96-100% ethanol** and **Magnetic Beads A.** We recommend a 10% overage to compensate for pipetting errors. Make sure to vortex the bulk mix before adding it to the lysate. It is recommended to prepare the bulk fresh every time a new DNA isolation is performed.
g. After 1 hour of incubation, the instrument will pause and will allow the user to add mixture of ethanol and magnetic beads prepared in **step f.** Open the cover and remove the plate from the instrument and add the prepared mixture from **step f** to **Column 1 and/or Column 7**.

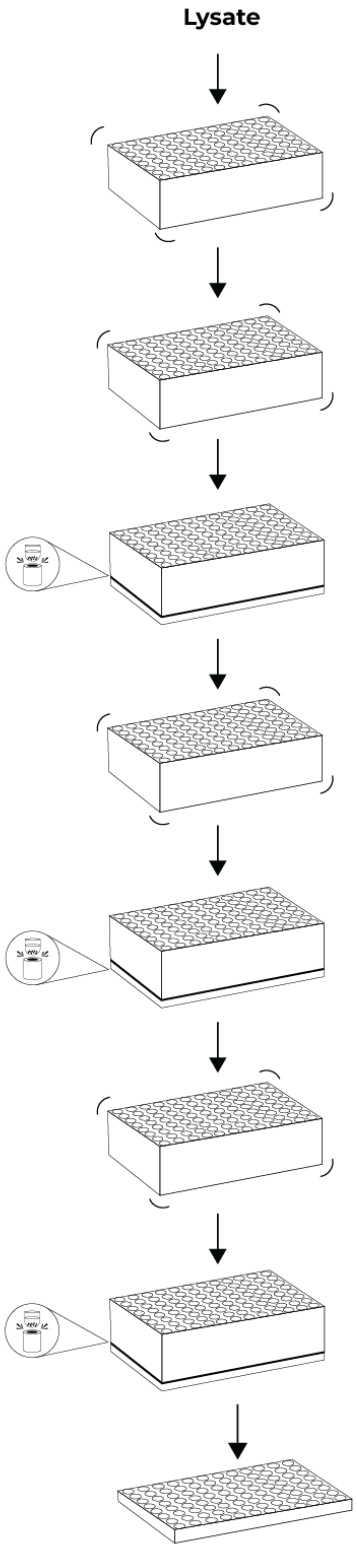
Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the lysate after addition of **96-100% ethanol** and **Magnetic Beads A** mixture to **Column 1 and/or 7** during step **f**. If RNase treatment is performed, make sure to use **CNTDNA16R** (IsoPure™ Mini) **program instead of CNTDNA16** to include an incubation step of 37°C for 15 minutes.

h. Put the plate back into the instrument and press OK. The instrument will continue to perform the extraction.
i. The instrument will prompt after the procedure is complete. Remove the plate from the instrument and the DNA is now ready for further downstream processing.

j. Transfer the eluted DNA from **Column 5 and/or Column 11** to a fresh plate. The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the plate for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

Flow Chart

Procedure for Norgen's Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) - Manual Method



II. Manual Genomic DNA Isolation from All Types of Lysate

Section 1. Lysate Preparation from various sample types

1A. Lysate Preparation from Animal Tissues

Notes Prior to Use

- Fresh or frozen tissues may be used for the procedure.
- Tissues should be immediately frozen and stored at -20°C or -70°C. Tissues may be stored at -70°C for several months.
- It is recommended that no more than 20 mg of tissue be used.

1A. Lysate Preparation from Animal Tissues

a) Excise up to 20 mg of tissue sample. Either frozen or fresh tissue may be used.
b) Homogenize the sample into a fine powder in liquid nitrogen using a mortar and pestle, then transfer into a nuclease-free microfuge tube.
c) Add 300 µL of the **Lysis Buffer B** to the tissue sample.

Note: The sample can also be homogenized using tools such as a rotor-stator homogenizer or a microfuge-size pestle in the provided Lysis Solution.

d) Use a syringe with a 22G needle to further homogenize the lysate by passing through the needle 10 times.
e) Transfer the lysate to the 96-Well Plate provided with the kit.
f) Add 20 µL of **Proteinase K** (vortex before use) to the lysate. Seal the plate with the Adhesive Tape (provided by user) and shake the plate in an orbital shaker at 1000 rpm for 2 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 2 minutes.
g) Incubate at 55°C for 1 hour.

Note: Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of tissue being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

h) Add 300 µL of **nuclease-free water.** Seal the plate with the Adhesive Tape (provided by user) and shake the plate in an orbital shaker at 1000 rpm for 2 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 2 minutes.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the lysate. Seal the plate with the Adhesive Tape (provided by user) and shake the plate in an orbital shaker at 1000 rpm for 2 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette. Incubate at 37°C for 15 minutes.

i) Remove the Adhesive Tape and add 600 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Beads A** (vortex prior to use) to the lysate collected above.
j) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
k) Proceed to **Section 2:** Genomic DNA Isolation from All Types of Lysate.

1B. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use

- Cells grown in suspension or monolayer may be used.
- The maximum recommended input of cells is 3 x 10^6.
- A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells.
- Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen cell pellets should not be subjected to repeated cycles of freeze and thaw prior to beginning the protocol.

1**B (i). Cell Lysate Preparation from Cells Growing in a Monolayer**

a) Detach cells by standard trypsinization method or cell scraper. Transfer an appropriate amount to a 1.5 mL microfuge tube (not provided). The maximum recommended input of cells is 3 x 10^6.
b) Collect cells by centrifugation at no more than 200 x g (~2,000 RPM) for 10 minutes. Discard the supernatant.
c) Add 100 µL of phosphate buffered saline (PBS) to the cell pellet. Mix by pipetting 10 times and transfer it to a 96-Well Plate (provided).
d) Add 20 µL of **Proteinase K** (vortex before use) to the lysate.
e) Add 300 µL of **Lysis Buffer B**.
f) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes
g) Incubate at 55°C for 20 minutes.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the lysate. Seal the plate with the Adhesive Tape (provided by user) and shake the plate in an orbital shaker at 1000 rpm for 2 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette. Incubate at 37°C for 15 minutes.

h) Remove the Adhesive Tape and add 400 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Beads A** (vortex prior to use) to the lysate.
i) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
j) Proceed to **Section 2:** Genomic DNA Isolation from All Types of Lysate**.**

1B (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

a) Transfer an appropriate amount of cells to a 1.5 mL microfuge tube (not provided). The maximum recommended input of cells is 3 x 10^6.
b) Collect cells by centrifugation at no more than 200 x g (~2,000 RPM) for 10 minutes. Discard the supernatant.
c) Add 100 µL of phosphate buffered saline (PBS) to the cell pellet. Mix by pipetting 10 times and transfer it to a 96-Well Plate (provided).
d) Add 20 µL of **Proteinase K** (vortex before use) to the lysate.
e) Add 300 µL of **Lysis Buffer B**.
f) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes
g) Incubate at 55°C for 20 minutes.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the lysate. Seal the plate with Adhesive Tape (provided by user) and shake the plate in an orbital shaker at 1000 rpm for 2 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette. Incubate at 37°C for 15 minutes.

h) Remove the Adhesive Tape and add 400 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Beads A** (vortex prior to use) to the lysate.
i) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
j) Proceed to **Section 2:** Genomic DNA Isolation from All Types of Lysate**.**

1C. Lysate Preparation for Viral DNA

Notes Prior to Use

- For the isolation of integrated viral DNA, follow **Section 1A** if the starting material is animal tissue and follow **Section 1B** if the starting material is cell culture.
- For the isolation of DNA from free viral particles, follow the protocol provided below.
- Up to 150 µL of viral suspension can be processed.
- Fresh samples are recommended. Frozen samples may be used, however the yield of genomic DNA may be decreased.

1C. Lysate Preparation for Viral DNA

a) To a 96-Well Plate (provided), add 20 µL of **Proteinase K** (vortex before use).
b) Transfer up to 150 µL of viral suspension to the 96-Well Plate.
c) Add 300 µL of **Lysis Buffer B**.
d) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
e) Incubate at 55°C for 1 hour.

Note: Incubation times may fluctuate between 45 minutes to over 2 hours depending on the type of cell being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 µL) to the lysate. Seal the plate with the Adhesive Tape (provided by user) and shake the plate in an orbital shaker at 1000 rpm for 2 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette. Incubate at 37°C for 15 minutes.

f) Remove the Adhesive Tape and add 450 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Beads A** (vortex prior to use) to the lysate.
g) Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
h) Proceed to **Section 2:** Genomic DNA Isolation from All Types of Lysate**.**

Section 2. Genomic DNA Isolation from All Types of Lysate

Note: The remaining steps of the procedure for the purification of genomic DNA are the same from this point forward for all the different types of lysate.

a. Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
b. Remove the Adhesive Tape. Aspirate and discard supernatant without touching the magnetic beads.
c. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of **Solution WN (ensure ethanol was added).**
d. Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 1 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minutes.
e. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
f. Aspirate and discard supernatant without touching the magnetic beads.
g. Add 500 µL of freshly prepared **70% ethanol**.
h. Seal the plate with an Adhesive Tape (provided by user) and mix the plate on an orbital shaker at 1000 rpm for 1 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minutes.
i. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
j. Aspirate and discard supernatant without touching the magnetic beads.
k. Repeat **Steps 2g – 2j** for a second wash step.

Note: Remove as much of the **70% ethanol** in the sample plate as possible by pipetting.

l. Incubate the 96-Well plate at 65˚C for 5 minutes to dry the magnetic beads. Make sure not to over-dry the magnetic beads as this might affect the DNA yield.
m. Add 50-100 µL of **Elution Buffer B**. Mix by gently pipetting and incubate at 65˚C for 10 minutes.
n. Briefly mix by pipetting and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
o. Carefully transfer the elution to a **96-Well Elution Plate** (provided) without touching the magnetic beads.
p. The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the **96-Well Elution Plate** for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidentally pipetted up with the supernatant.	The pipette tip was placed too close to the magnetic beads while pipetting	Return the magnetic beads and the supernatant back into the sample tube. Mix well, and place the tube back onto the magnetic separation rack for the specified time. Carefully remove the supernatant without touching the magnetic beads.
The yield of genomic DNA is low	Incomplete lysis of cells	Ensure that correct lysis protocol was applied to the sample. Ensure Proteinase K is added properly.
	Amount of magnetic beads added was not sufficient	Ensure that the Magnetic Beads A is mixed well prior to use to avoid any inconsistency in DNA isolation.
	DNA concentration in the cell or tissue sample being used is low.	Some samples contain very little target DNA. This varies from individual to individual based on numerous variables. Extend the incubation time of Proteinase K digestion or reduce the amount of tissue or cells used for lysis.
Very gelatinous prior to adding the Magnetic bead and Ethanol	The lysate solution mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before adding the magnetic beads to the lysate.
	Maximum number of cells or	Refer to specifications to determine if amount of starting

	amount of tissue exceeds kit specifications	material falls within kit specifications.
DNA does not perform well in downstream applications.	DNA was not washed with ethanol	Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with 70% Ethanol. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads.
	Ethanol carryover	Ensure that the drying step after the 70% ethanol wash steps is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is present in eluted DNA.	RNA is co-eluted with the DNA	Carry out a digestion with RNase A on the elution if the RNAse present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

Related Products	**Product #**
Cells and Tissue DNA Isolation Kit (Magnetic Bead System)	59100
Cells and Tissue DNA Isolation Kit (50 Prep)	53100
Bacterial Genomic DNA Isolation Kit	17900
Blood DNA Isolation Mini Kit	46300
Adhesive Tape – 100 Units	28394

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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