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Cells and Tissue DNA Isolation Micro Kit — Product Insert

Product # 57300

Norgen's **Cells and Tissue DNA Isolation Micro Kit** is designed for the rapid preparation of genomic DNA from low inputs of cultured cells as well as various tissue samples and urine. Purification is based on spin column chromatography as the separation matrix. Norgen's columns bind DNA under optimized salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with PCR and Southern Blot analysis.

The Cells and Tissue DNA Isolation Micro Kit is optimized to isolate DNA from small inputs of cells and tissues, such as Laser-Captured Microdissection (LCM) samples. The genomic DNA is preferentially purified from other cellular proteinaceous components, and is eluted into a small elution volume. Typical yields of genomic DNA will vary depending on the sample being processed. Preparation time for a single sample is approximately 60 minutes, and each kit contains sufficient materials for 50 preparations.

Kit Components

Component	Product # (50 samples)
Lysis Buffer B	20 mL
Solution WN	18 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Proteinase K	1.2 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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Advantages

- Optimized for small inputs of cells and tissues, such as Laser-Captured Microdissection (LCM)
- DNA is eluted in small elution volumes (20 – 40 µL)
- Fast and easy processing using a rapid spin-column format
- Isolate high quality genomic DNA with no RNA or protein contamination
- Recovered genomic DNA is compatible with various downstream applications

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.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for 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.

The **Lysis Buffer B** and **Solution WN** contain guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 2 mL microcentrifuge tubes
- Nuclease-free water
- Phosphate buffered saline (PBS)
- 96 - 100% ethanol
- 56°C water bath or incubator
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer
- Syringe with a 22G needle
- RNase A (if RNA-free DNA is required)
- Lysozyme (400 mg/mL) (Required only for lysate preparation from Urine Bacterial Cells)

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Section 1. Preparation of Lysate From Various Cell Types

Notes Prior to Use:

- 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.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- 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.
- Prepare a working concentration of the **Solution WN** by adding 24 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution I. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution II. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Preheat a water bath or heating block to 56°C.
- Always vortex the Proteinase K before use.
- RNase A (not provided) can be used if RNA-free DNA is required. Use RNase A at the indicated optional step in the protocol.
- For Lysate Preparation from Urine Bacterial Cells: Prepare a 400 mg/mL stock solution (approximately 1.7 x10^7 units/mL) of lysozyme as per supplier's instructions.

Flow Chart

Procedure for Purifying Genomic DNA using Norgen's Cells and Tissue Genomic DNA Isolation Micro Kit

Obtain cells or tissue sample

Add Lysis Buffer B and Proteinase K. Vortex. Incubate.

Add ethanol. Vortex.

Bind to column

SPIN

Wash once with Solution WN.
Wash twice with Wash Solution A.
Dry spin.

SPIN

Elute DNA with Elution Buffer B

SPIN

Pure Genomic DNA

1A. Lysate Preparation from Animal Tissues

Notes Prior to Use

- Norgen's Cells and Tissue DNA Isolation Micro Kit is designed for isolating DNA from small amounts of tissue samples. It is recommended that no more than 3 mg of tissue be used, in order to prevent clogging of the column. If a larger amount of starting material is desired please use Norgen's Cells and Tissue DNA Isolation Kit (Cat. # 53100), which can be used to process up to 20 mg of tissue.
- 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.

1A. Lysate Preparation from Animal Tissues

a. Excise up to 3 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. Ensure efficient homogenization to enhance tissue lysis and reduce the risk of column clogging.

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. Incubate at 56°C for 1 hour.
g. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
h. Add 300 µL of nuclease-free water. Mix by vortexing.
i. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

j. Add 110 µL of ethanol. Mix by vortexing.
k. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
l. Proceed to Step 2: Binding to Column.

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 5×10^5. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, each well of a confluent 12-well plate of HeLa cells will contain 5×10^5 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.** Detach cells using a 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 5 x 10^5.
- **b.** Collect cells by centrifugation at no more than 200 *x g* (~2,000 RPM) for 10 minutes. Discard the supernatant
- **c.** Add 200 µL of phosphate buffered saline (PBS) to the cell pellet. Mix by gentle vortexing.
- **d.** Add 20 µL of **Proteinase K** (vortex before use) to the lysate.
- **e.** Add 300 µL of **Lysis Buffer B.** Mix well by vortexing.
- **f.** Incubate at 56°C for 20 minutes.
- **g.** Briefly spin the tube to collect any drops of liquid from the inside of the lid.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

- **h.** Add 110 µL of ethanol. Mix by vortexing.
- **i.** Briefly spin the tube to collect any drops of liquid from the inside of the lid.
- **j.** Proceed to Step 2: Binding to Column.

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 5 x 10^5.
- **b.** Collect cells by centrifugation at no more than 200 *x g* (~2,000 RPM) for 10 minutes. Discard the supernatant.
- **c.** Add 200 µL of phosphate buffered saline (PBS) to the cell pellet. Mix by gentle vortexing.
- **d.** Add 20 µL of **Proteinase K** (vortex before use) to the lysate.
- **e.** Add 300 µL of **Lysis Buffer B.** Mix well by vortexing.
- **f.** Incubate at 56°C for 20 minutes.
- **g.** Briefly spin the tube to collect any drops of liquid from the inside of the lid.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

- **h.** Add 110 µL of ethanol. Mix by vortexing.
- **i.** Briefly spin the tube to collect any drops of liquid from the inside of the lid.
- **j.** Proceed to Step 2: Binding to Column.

1C. Lysate Preparation from Laser-Captured Microdissection (LCM)

Notes Prior to Use

- LCM samples obtained from frozen sections are recommended. Formalin-Fixed, Paraffin-Embedded sections may also be used. However, DNA isolated from FFPE samples generally has poorer quality than that from frozen sections.

1C. Cell Lysate Preparation from Laser-Captured Microdissection (LCM)

- **a.** Aliquot 300 µL of **Lysis Buffer B** to a microcentrifuge tube.
- **b.** Remove the thermoplastic film containing the captured cells using sterile fine forceps. Carefully submerge the sample into the **Lysis Buffer B**.
- **c.** Add 20 µL of **Proteinase K** (vortex before use) to the suspension. Mix well by vortexing.

d. Incubate the sample at 56ºC for 30 minutes. Apply vortex for 15 seconds after every 10 minutes.
e. At the end of the incubation, vortex the tube one more time for 15 seconds. The thermoplastic film may be removed at this point using sterile fine forceps. Otherwise, proceed to **Step 1Cf.**

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

k. Add 55 µL of ethanol. Mix by vortexing.
l. Proceed to Step 2: Binding to Column.

1D. 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.

1D. Lysate Preparation for Viral DNA

a. To a 1.5 mL microfuge tube (not provided), add 20 µL of **Proteinase K** (vortex before use).
b. Transfer up to 150 µL of viral suspension to the tube.
c. Add 300 µL of **Lysis Buffer B.** Mix well by vortexing.
d. Incubate at 56°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.

e. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

f. Add 110 µL of ethanol. Mix by vortexing.
g. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
h. Proceed to Step 2: Binding to Column.

1E. Lysate Preparation from Urine Exfoliated Cells

Notes Prior to Use

- It is recommended that no more than 25 mL of urine be used for each column.
- It is recommended that at least 1 mL of urine is used for each isolation.

1E. Lysate Preparation from Urine Exfoliated Cells

a. Transfer 1 - 1.5 mL of urine to a microcentrifuge tube and centrifuge at 2,000 RPM for 5 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

Note: For urine samples larger than 1.5 mL a swinging bucket centrifuge can be used to pellet the cells at 2,000 RPM for 5 minutes. The maximum input of urine is 25 mL or 1 x 10^6 cells per column. We recommend that the cell count be determined using standard cytological methods.

b. Add 200 µL of phosphate buffered saline (PBS) to the cell pellet. Resuspend the cells by gentle vortexing.
c. Add 20 µL of **Proteinase K** (vortex before use) to the lysate.
d. Add 300 µL of **Lysis Buffer B.** Mix well by vortexing.
e. Incubate at 56°C for 15 minutes.
f. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

g. Add 110 µL of ethanol. Mix by vortexing.
h. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
i. Proceed to Step 2: Binding to Column.

1F. Lysate Preparation from Urine Bacterial Cells

Notes Prior to Use

- It is recommended that no more than 25 mL of urine be used for each column.
- It is recommended that at least 1 mL of urine is used for each isolation.

1F. Lysate Preparation from Urine Bacterial Cells

a. Transfer 1 - 1.5 mL of urine to a microcentrifuge tube and centrifuge at 14,000 RPM for 3 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

Note: For urine samples larger than 1.5 mL a swinging bucket centrifuge can be used to pellet the cells at 3,000 x g for 5 minutes. The maximum input of urine is 25 mL.

b. Add 200 µL of phosphate buffered saline (PBS) to the cell pellet. Resuspend the cells by gentle vortexing.
c. Add 20 µL of **Proteinase K** (vortex before use) to the cell suspension.
d. Add 12 µL of previously prepared lysozyme stock solution.
e. Add 300 µL of **Lysis Buffer B.** Mix well by vortexing.
f. Incubate at 56°C for 30 minutes.
g. Briefly spin the tube to collect any drops of liquid from the inside of the lid.

Optional RNase A Treatment:
If RNA-free genomic DNA is required, add the equivalent of 500 units of RNase A (not to exceed 10 µL) to the lysate. Mix well and incubate at 37°C for 15 minutes.

h. Add 110 µL of ethanol. Mix by vortexing.
i. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
j. Proceed to Step 2: Binding to Column.

Section 2. Genomic DNA Purification 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.

2. Binding to Column

a. Assemble a **Micro Spin Column** with a provided collection tube. Apply up to 750 µL of the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 2 minutes at 6,000 *x g* (~ 8,000 RPM).

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin at 14,000 *x g* (~14,000 RPM) for 2 minutes.

b. After centrifugation, discard the flowthrough, and reassemble the spin column with its collection tube.

c. Repeat Step **2a** and Step **2b** until all the lysate mixture has passed through the column

3. Washing Bound DNA

a. Apply 500 µL of **Solution WN** (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Apply 500 µL of **Wash Solution A** (ensure ethanol was added) to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

c. Wash column another time by adding 500 µL of **Wash Solution A** and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

d. Spin the column for 2 minutes in order to thoroughly dry the column at 14,000 x g (~14,000 RPM). Discard the collection tube.

4. Elution of Clean DNA

a. Place the column into a provided 1.7 mL elution tube.

b. Add 40 µL of **Elution Buffer B** to the column.

Note: For higher concentrations of DNA, a lower elution volume may be used. A minimum volume of 20 µL is recommended.

c. Incubate at room temperature for 1 minute.

d. Centrifuge for 1 minute at 6,000 x g (~8,000 RPM) followed by 1 minute at 14,000 x g (~14,000 RPM).

(Optional): An additional elution may be performed if desired by repeating steps **4a – 4d**. Collect second elution into a new microcentrifuge tube.

5. Storage of DNA

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.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The micro spin column is clogged	The sample is too large	Do not exceed the recommended amount of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. Clogging can also be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
The lysate is very gelatinous prior to loading onto the column	The lysate solution mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
The yield of genomic DNA is lower than was expected based on input	Improper storage of samples	Tissue samples and cell pellets may be frozen and stored at -20°C or -70°C. Repeated freezing and thawing of stored samples should be avoided, as this may lead to decreased yields of DNA.
	Incomplete lysis of cells	Ensure efficient homogenization of tissue samples and extend the incubation time of Proteinase K digestion or reduce the amount of tissue or cells used for lysis.
The genomic DNA is sheared	The genomic DNA was handled improperly	Pipetting steps should be handled as gently as possible. Reduce vortexing times during mixing steps (no more than 10-15 seconds).
	Improper storage of sample	Repeated freezing and thawing of stored samples should be avoided as this may lead to decreased DNA size.
	The sample is old	Sheared DNA may be obtained from old tissue or cell samples. Fresh samples are recommended for maximum genomic DNA yield
DNA does not perform well in downstream applications.	DNA was not washed with the provided solutions	Ensure the column was washed once with Wash Solution WN and twice with Wash Solution A.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
Cells and Tissue DNA Isolation Kit	53100
Bacterial Genomic DNA Isolation Kit	17900
Blood Genomic DNA Isolation Mini Kit	46300
RNase A	26260

Technical Support
Contact our Technical Support Team between the hours of 8:30 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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