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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, cultured cells, 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. The Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System) also can be integrated with a robotic automation system.

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 Bead Suspension and ethanol are then added to the clean supernatant, and the resulting solution is placed on the magnetic separation rack. 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	50 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 Bead Suspension	8.5 mL
96-Well Plate	2
96-Well Elution Plate	2
Adhesive Tape	2
Product Insert	1

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature (15 – 25°C). These reagents should remain stable for at least 2 years in their unopened containers.

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 saliva.

Customer-Supplied Reagents and Equipment
- Magnetic Bead 96-Well Separation Plate
- 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)
- RNase A (optional)

Procedure

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.
- 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 Bead Suspension** 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).
- 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 Purifying Cells and Tissue DNA using
Norgen's Cells and Tissue DNA Isolation 96-Well Kit (Magnetic Bead System)

Prepare lysate by adding Lysis Buffer B and Proteinase K to sample

Incubate at 55ºC

Transfer

Add ethanol and Magnetic Bead Suspension.
Mix.
Incubate for 5 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Discard supernatant. Add Solution WN,
mix and incubate for 1 minute.

Place onto magnetic plate.
Let stand for 1 minute.

Discard supernatant. Add 70% ethanol,
mix and incubate for 1 minute.

Repeat ethanol wash step.
Incubate open tube at 65˚C for 5 minutes.

Add Elution Buffer B, mix and
Incubate at 65˚C for 10 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Carefully transfer supernatant
to Elution Tube.

Pure DNA

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, in order to prevent clogging of the column.

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. Add 20 µL of **Proteinase K** (vortex before use) to the lysate. Mix well by gentle vortexing.

f. 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.

g. Add 300 µL of nuclease-free water. Mix by vortexing.

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. Mix well and incubate at 37°C for 15 minutes.

h. Transfer lysate into the 96-Well Plate and add 600 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by gentle pipetting.

i. Incubate at room temperature for 5 minutes.

j. 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 3×10^6. 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. 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 3×10^6.

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 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 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. Mix well and incubate at 37°C for 15 minutes.

g. Transfer lysate to the 96-Well Plate and add 400 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above.
h. Incubate at room temperature for 5 minutes. Occasionally shake the plate.
i. Proceed to Step 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 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 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. Mix well and incubate at 37°C for 15 minutes.

g. Transfer lysate to the 96-Well Plate and add 400 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by gentle pipetting.
h. Incubate at room temperature for 5 minutes. Occasionally invert the tube.
i. Proceed to Step 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 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 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. Mix well and incubate at 37°C for 15 minutes.

e. Transfer lysate to the 96-Well Plate and add 450 µL of **96 – 100% ethanol** and 40 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by gently pipetting.
f. Incubate at room temperature for 5 minutes. Occasionally invert the tube.
g. Proceed to Step 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.
b. 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). Resuspend by pipetting and incubate at room temperature for 1 minute.
d. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
e. Aspirate and discard supernatant without touching the magnetic beads.
f. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of freshly prepared **70% ethanol**. Resuspend by pipetting and incubate at room temperature for 1 minute.
g. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
h. Aspirate and discard supernatant without touching the magnetic beads.
i. Repeat **Steps 2f - 2h** for a second wash step.

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

j. Incubate the 96-Well plate at 65˚C for 5 minutes to dry the magnetic beads.
k. Add 100-200 µL of **Elution Buffer B**. Mix by gently pipetting and incubate at 65˚C for 10 minutes.
l. Briefly mix by pipette and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
m. Carefully transfer the elution to a 96-Well Elution Plate (provided) without touching the magnetic beads. The purified DNA sample may be stored at 4°C for a few days. The provided adhesive tape can be used for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

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

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.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidently 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 well. Mix well, and place the plate back onto the magnetic separation plate 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 bead suspension 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 amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
DNA does not perform well in downstream applications.	DNA was not washed with 70% 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 coeluted 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.

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