Genomic DNA Isolation Kit

Product # 24700, 24750, 24770

Product Insert

Norgen's **Genomic DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from various tissue samples, cultured cells, bodily fluids and nasal or throat swabs. Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds DNA under high 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 Genomic DNA Isolation Kit allows for the isolation of genomic DNA from various types of animal tissues or cell samples. In addition, a protocol is also provided for the purification of viral DNA. In all cases the genomic DNA is preferentially purified from other cellular proteinaceous components. 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 # 24700 (50 samples)	Product # 24750 (100 samples)	Product # 24770 (250 samples)
Digestion Buffer A	25 mL	2 x 25 mL	5 x 25 mL
Buffer SK	30 mL	2 x 30 mL	5 x 30 mL
Wash Solution A	18 mL	2 x 18 mL	5 x 18 mL
Elution Buffer B	30 mL	2 x 30 mL	5 x 30 mL
Proteinase K	12 mg	2 x 12 mg	5 x 12 mg
Spin Columns	50	100	250
Collection Tubes	50	100	250
Elution tubes (1.7 mL)	50	100	250
Product Insert	1	1	1

Storage Conditions and Product Stability

The Proteinase K should be stored at -20°C upon arrival and after reconstitution. All other solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

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*.

Buffer SK contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of this solution.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 55°C water bath or heating block
- 96 – 100% ethanol
- Cell Disruption Tools such as mortar and pestle, rotor-stator homogenizer
- RNase A (optional)

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 **(Step 1)**. However, the subsequent steps are the same in all cases **(Steps 2 – 4)**.
- 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.
- Reconstitute each vial of Proteinase K in 0.6 mL of molecular biology grade water, aliquot in 120 µL fractions and store the unused portions at -20°C until needed.
- Add 42 mL of 96-100% ethanol to all the provided bottles of **Wash Solution A**. 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 55°C.

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. Place the sample in a nuclease-free microfuge tube.

Note: The tissue sample may be homogenized into a fine powder in liquid nitrogen to improve lysis efficiency

b. Add 300 µL of **Digestion Buffer A** to the tissue sample. Homogenize the sample using tools such as a rotor-stator homogenizer or a microfuge-size pestle.

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 cell suspension. Mix well.

c. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and 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.

d. Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing
e. Add 300 µL of 96 – 100% ethanol. Mix by vortexing.
f. 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 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. 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 200 µL of **Digestion Buffer A** to the cell pellet. Mix by gentle 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 cell suspension. Mix well.

d. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and 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.

e. Add 200 µL of **Buffer SK** to the lysate. Mix by vortexing
f. Add 200 µL of 96 – 100% ethanol. Mix by vortexing.
g. 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 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 200 µL of **Digestion Buffer A** to the cell pellet. Mix by gentle 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 cell suspension. Mix well.

d. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and 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.

e. Add 200 µL of **Buffer SK** to the lysate. Mix by vortexing
f. Add 200 µL of 96 – 100% ethanol. Mix by vortexing.
g. Proceed to Step 2: Binding to Column.

1C. Lysate Preparation from Bodily Fluids or Swabs

Notes Prior to Use

- Up to 150 µL of bodily fluids including blood and saliva can be processed.
- Fresh samples of bodily fluids are recommended. Frozen samples may be used, however the yield of genomic DNA may be decreased.

1C. Lysate Preparation from Bodily Fluids or Swabs

a. *For Bodily Fluid*: Transfer up to 150 µL of a bodily fluid sample to a 1.5 mL microfuge tube (not provided). Adjust the volume to 300 µL by adding **Digestion Buffer A**. Proceed to Step **1Cc**.
b. *For Swabs*: Using sterile techniques, cut the cotton tip where the cells were collected and place into 300 µL of **Digestion Buffer A** in a microcentrifuge tube. Vortex gently and incubate for 5 minutes at room temperature. Remove the cotton tip with sterile forceps. Alternatively, transfer the cells (in **Digestion Buffer A**) to a 1.5 mL microcentrifuge tube (not provided). Proceed to Step **1Cc**.
c. 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 cell suspension. Mix well.
d. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and 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.

e. Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing
f. Add 300 µL of 96 – 100% ethanol. Mix by vortexing.
g. 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, follow Section **1B** if the starting material is cell culture, and follow Section **1C** if the starting material is bodily fluid such as blood.
- For the isolation of DNA from free viral particles, follow the protocol provided below.
- Up to 150 µL of viral suspension or bodily fluids 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. Transfer up to 150 µL of a sample to a 1.5 mL microfuge tube (not provided). Adjust the volume to 300 µL by adding **Digestion Buffer A**.

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 cell suspension. Mix well.

b. Add 12 µL of **Proteinase K** to the suspension. Mix well by gentle vortexing and 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.

c. Add 300 µL of **Buffer SK** to the lysate. Mix by vortexing
d. Add 300 µL of 96 – 100% ethanol. Mix by vortexing.
e. 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 spin column with a provided collection tube. Apply up to 600 µL of the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 3 minutes at 5,200 *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 **Wash Solution A** to the column, and centrifuge the unit for 1 minute at 14,000 *x g* (~14,000 RPM).
b. After centrifugation, discard the flowthrough and reassemble the spin column with its collection tube.
c. Apply 500 µL of **Wash Solution A** to the column, and centrifuge the unit for 2 minutes at 14,000 *x g* (~14,000 RPM).
d. Carefully detach the spin column from the collection tube and discard the collection tube and flowthrough.

Note: If any liquid is left on the side of the spin column, discard the flowthrough and reassemble the spin column with its collection tube. It is highly recommended to spin for an additional 1 minute at 14,000 *x g* (~14,000 RPM) in order to completely dry the column.

4. Elution of Clean DNA

a. Assemble the spin column (with DNA bound to the resin) with a provided 1.7 mL Elution tube.
b. Add 200 µL of **Elution Buffer B** to the center of the resin bed. Centrifuge for 1 minute at **3,000 *x g* (~6,000 RPM)**. A portion of the **Elution Buffer B** will pass through the column which allows for hydration of the DNA to occur.
c. Centrifuge at **14,000 *x g* (~14,000 RPM)** for an additional 2 minutes to collect the total elution volume.
d. **(Optional):** An additional elution may be performed if desired. Another 200 µL of **Elution Buffer B** may be added to the column and centrifuged at 3,000 *x g* for 1 minute into a new elution tube. Then, centrifuge the column at 14,000 *x g* for an additional 2 minutes. The yield can be improved by an additional 20-30% when this second elution is performed.

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

Related Products	Product #
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100
Bacterial Genomic DNA Isolation Kit	17900

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
The 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/binding 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 low	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	Extend the incubation time of Proteinase K digestion or reduce the amount of tissue or cells used for lysis.
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 *x g* is performed after the 3,000 *x g* centrifugation cycle, to ensure that all the DNA is eluted.
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

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