

Urine DNA Isolation Kit

Product #18100

Product Insert

Norgen's **Urine DNA Isolation Kit** provides a fast and simple procedure for isolating DNA from 2 mL of urine. DNA found in urine can be divided into 2 basic categories. The larger species is generally greater than 1 kb in size, and appears to be derived mainly from cells shed into the urine from the urinary tract. The second species is smaller, generally between 150 and 250 bp, and derives, at least in part, from the circulation. Both types of DNA can be isolated using this kit.

With Norgen's Urine DNA Isolation Kit, purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Typical yields of DNA isolated using the Norgen Urine DNA Isolation Kit will vary depending on the input sample, with more concentrated samples tending to yield more DNA. Preparation time for a single sample is less than 90 minutes. The process does not require ethanol or other alcohols. The purified urine DNA is compatible with PCR and Southern Blot analysis. The kit has a shelf life of at least 1 year when stored as suggested.

Kit Components

Component	Product #18100 (50 preps)
Stabilizer	3 mL
Activation Buffer	60 mL
Binding Solution I	25 mL
Binding Solution II	15 mL
Incubation Solution	60 mL
Elution Buffer	8 mL
Wash Solution	60 mL
Micro Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
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Storage Conditions and Product Stability

All 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.

The **Binding Solution II**, **Incubation Solution** and **Wash Solution** contain 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 these solutions.

The **Stabilizer** contains sodium azide. Sodium azide is highly toxic, and may be fatal if swallowed or absorbed through skin. Careful handling of this material must be followed, and should include the use of protective eye wear, gloves and lab coats.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- Micropipettors
- 60°C incubator
- 15 mL tubes

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. All centrifugation steps are performed at 3,300 x g unless otherwise stated, and at room temperature. Centrifugation at 4°C will not adversely affect kit performance. 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.

Notes prior to use:

- 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.
- Preheat an incubator or heating block to 60°C.

1. Urine Sample Collection and pH Adjustment

- a. Collect 2 mL of urine sample into a sterile 15 mL tube. Stabilizer should be added to the urine sample immediately upon collection. Add 20 μL of Stabilizer for every 2 mL urine sample.

Note: If the urine sample is not going to be processed within a day of collection, protease inhibitors must be added. We recommend that Sigma's Protease Inhibitor Cocktail is used (Product Number P2714). This product contains a mixture of protease inhibitors known to be very effective with our kit. The cocktail includes AEBSF, EDTA, Bestatin, E-64, Leupeptin and Aprotinin. Add 20 μL of this cocktail to the 2 mL sample of urine, as per manufacturer's instructions.

- b. Add 80 μL of **Binding Solution I** to the 2 mL urine sample in order to adjust the pH to 3.5. Verify the pH of the sample, and add more Binding Solution I if necessary. Some samples may require more than 80 μL of Binding Solution I.

Note: In some concentrated urine samples, precipitation may occur with the addition of Binding Solution I. This precipitate includes urine proteins and DNA, and thus should not be discarded. The precipitate should be resuspended as much as possible and loaded onto the column with the rest of the sample.

- c. Add 250 μL of **Binding Solution II** to the pH adjusted urine sample.
- d. Mix contents well.

2. Column Activation

- a. Assemble a spin column with a provided collection tube.
- b. Add 500 μL of **Activation Buffer** to the column and close the cap.
- c. Centrifuge for 2 minutes and discard the flowthrough.
- d. Repeat steps **b** and **c** to complete the Column Activation Step.

3. Sample Binding to Column

- a. Apply 650 μL of the pH-adjusted urine sample onto the column and centrifuge for 3 minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Repeat steps **a** and **b** until the entire urine sample has been loaded, in order to complete the Sample Binding Step.

Note: If the urine sample is not passing through the column easily, the speed of centrifugation may be increased to 6,700 x g. **Do not** increase the centrifugation speed beyond 6,700 x g, as this promotes DNA loss.

4. Column Incubation

- a. Add 650 μL of **Incubation Solution** to the column and centrifuge for 2 minutes.

Note: If the centrifugation speed had to be increased to 6,700 x g for the Binding Step, then the column will have to be spun at this speed for this step.

- b. Retain the flowthrough in the collection tube and place the column and collection tube in a 60°C incubator for **1 hour**.

Note: A small amount of liquid may remain on the column after centrifugation. This should be left on the column, and incubated as stated above. Also, increasing the incubation time to >2 hours will increase the amount of DNA recovered.

- c. After 1 hour, place the flowthrough back onto the column and centrifuge for 2 minutes.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.

5. Column Wash

- a. Apply 500 μL of **Wash Solution** to the column and centrifuge for 2 minutes.
- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Apply another 500 μL of **Wash Solution** to the column and centrifuge for 2 minutes.
- d. Inspect the column to ensure that all the liquid has passed through into the collection tube. If necessary, spin for an additional minute or two to dry.

6. DNA Elution

- a. Transfer the spin column to a fresh 1.7 mL Elution tube.
- b. Apply 50 μL of **Elution Buffer** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM)**, followed by **1 minute at 14,000 x g (~14,000 RPM)**.

Urine DNA samples are now ready for downstream applications.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The micro spin column is clogged.	Centrifugation speed was too low or spin time was inadequate.	Check the centrifuge to ensure that it is capable of generating 3,300 x g. Sufficient centrifugal force is required to move the liquid phase through the resin. Also ensure that the correct spin times are followed. Centrifugation speeds may be increased to 6700 x g if needed.
	Sample is too concentrated.	If there is too much protein present in the sample, then the column may become clogged. In this case, centrifugation speeds may be increased to 6700 x g, but this speed should not be exceeded for the Sample Binding step. The centrifugation time may also be increased to pass all the liquid through the column.
A precipitate forms in the sample prior to binding.	The mixture of urine sample and Binding Buffers is not homogeneous.	The precipitate should not be discarded. Resuspend the precipitate as much as possible and load it onto the column with the rest of the sample.
The yield of DNA is low.	Urine sample DNA concentration too low.	Some urine samples contain very little DNA. This varies from individual to individual based on numerous variables. In order to increase the yield, the amount of urine input onto the column could be increased or several columns could be processed in tandem. Increasing the incubation time at 60°C (up to overnight) could also result in increased yields. Alternatively, one could use the Proteospin™ Urine Concentration Kit to concentrate the proteins and then use the elution of that kit combined with the 2mL urine sample to proceed with the current kit.
DNA does not perform well in downstream applications.	A different Elution Buffer was used.	If a different Elution Buffer was used other than the one provided in the kit, the buffer should be checked for any components that may interfere with the application. Common components that are known to interfere are high salts (including EDTA), detergents and other denaturants. Check the compatibility of your elution buffer with the intended use.

Related Products	Product #
ProteoSpin™ Urine Protein Concentration Micro Kit	17400

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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