

Urine DNA Isolation Kit Dx (Slurry Format)

REF Dx48800



IVD

Product Insert

 PIDx48800-4

Intended Use

Norgen's Urine DNA Isolation Kit Dx (Slurry Format) provides a fast, reliable and simple procedure for isolating DNA from various amounts of urine ranging from 3 mL to 25 mL for subsequent *in vitro* diagnostic use. Both human and viral DNA can be isolated using this kit.

This kit is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated using the DNA isolated with Norgen's Urine DNA Isolation Kit Dx (Slurry Format) in conjunction with an *in vitro* diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, suitable controls for downstream applications should be used.







Norgen's Urine DNA Isolation Kit Dx (Slurry Format) is intended for use by professional users such as technicians, physicians and biologists experienced and trained in molecular biological techniques including DNA isolation.

Norgen's Urine DNA Isolation Kit Dx (Slurry Format) does not provide a diagnostic result. It is the sole responsibility of the user to use and validate the kit in conjunction with a downstream *in vitro* diagnostic assay.

Kit Components

| Component | Product #Dx48800 (50 samples) |
|--------------------------|----------------------------------|
| Solution A | 18 mL |
| Solution B | 30 mL |
| Wash Solution | 22 mL |
| Elution Buffer | 6 mL |
| Mini Filter Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
| Product Insert | 1 |

Label Legend

| | | | | | | | | |
|---|---|------------|------------------|---|---|------------------------------------|---|---|
|  |  | LOT | REF |  |  | IVD |  |  |
| Do not reuse | Use by | Batch Code | Catalogue Number | Contains sufficient for <n> tests | Manufacturer | In Vitro Diagnostic Medical Device | Consult instructions for use | Temperature limitation |

Advantages

- CE-IVD marked in accordance with Regulation (EU) 2017/746
- Fits into *in vitro* diagnostic workflows
- Sample collection is non-invasive and painless
- Fast and easy processing using a rapid spin-column format
- Isolate high quality genomic DNA
- Compatible with preserved urine samples collected using Norgen's Urine Preservative (please see Related Products Table)

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment. It is recommended to warm Solution A and Solution B for 20 minutes at 60°C if any salt precipitation is observed.

Precautions

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 **Solution B** 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.

Urine 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 urine.

Should any serious incident, such as injury or impairment of the user, occur during the use of this device, or because the use of this device, please contact Norgen Biotek to report the incident. Additionally, report the incident to the competent authority in the country in which the user is located.

Customer-Supplied Reagents and Equipment

- Centrifuge with a swinging bucket rotor capable of 2000 RPM
- Benchtop microcentrifuge
- Micropipettors
- 96 – 100% ethanol
- 60°C incubator
- 15 mL tubes
- 50mL tubes
- Lysozyme Stock Solution (400 mg/mL)
- Proteinase K (20 mg/mL)

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:

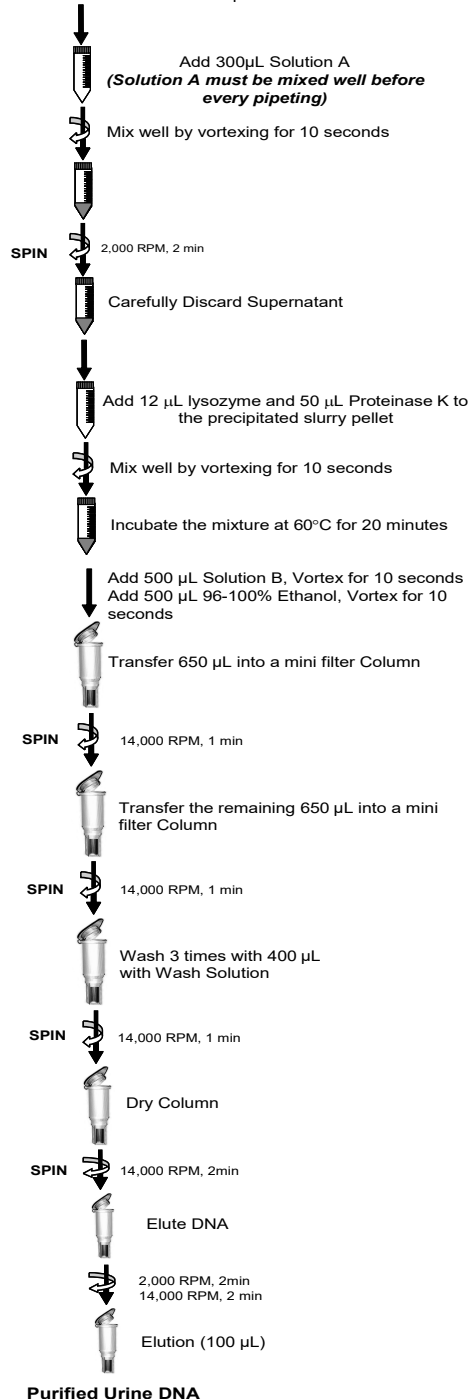
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

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

Flow Chart

Procedure for Purifying Urine DNA using Norgen's Urine DNA Isolation Kit (Slurry Format) Dx

In 50mL tube add 3-25mL urine Sample



Notes Prior to Use

- We recommend the use of **Norgen's Urine Preservative** when collecting urine samples, which is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures. The components of the Urine Preservative allow samples to be stored for over 2 years at room temperature with no detected degradation of urine DNA, RNA or proteins. Norgen's Urine Preservative is available in 2 convenient formats: in a liquid format in Norgen's Urine Preservative Single Dose Ampules, as well as in a dried format in Norgen's Urine Collection and Preservation Tubes. Please see the Related Products table below.
- Do not spin down or filter the urine sample before proceeding with the isolation, as this could decrease the DNA yield.
- 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 400 mg/mL stock solution (approximately 1.7×10^7 units/mL) of lysozyme as per supplier's instructions
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of the Wash Solution by adding 50 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Detailed Procedure

1. Add 300 μ L of **Solution A** to each urine sample. Mix well by vortexing for 10 seconds.
(Note 1: Solution A must be mixed well before every pipeting)
(Note 2: The volume of Solution A is fixed with all urine volumes ranging from 3 mL and up to 25 mL)
2. Centrifuge for **2 minutes at 2,000 RPM**, then discard the supernatant carefully in order not to dislodge the precipitated slurry pellet.
3. Add 12 μ L of the previously prepared lysozyme and 50 μ L of Proteinase K (20 mg/mL) to the precipitated slurry pellet. Vortex for 10 seconds. Incubate the mixture at 60°C for 20 minutes.
4. Add 500 μ L **Solution B** to the precipitated slurry pellet, mix well by vortexing for 10 seconds.
5. Add 500 μ L of **96-100% Ethanol** to the mix from **Step 4**, mix well by vortexing for 10 seconds.
6. Transfer 650 μ L from the previous mix into a Mini Filter Spin column and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Repeat **Step 6** until the entire mixture from **Step 5** has been transferred to the Mini Filter Spin Column.
8. Apply 400 μ L of **Wash Solution** to the column and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.
9. Repeat Step 8 to wash column second time.
10. Wash the column a third time by adding another 400 μ L of **Wash Solution** and centrifuge for **1 minute** at 14,000 RPM. Discard the flowthrough and reassemble the spin column with its collection tube.
11. Spin the column for **2 minutes** empty at 14,000 RPM in order to thoroughly dry the resin. Discard the collection tube.

12. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 μ L of **Elution Buffer** to the column and centrifuge for **2 minutes at 2,000 RPM**, followed by **2 minutes at 14,000 RPM**.

Frequently Asked Questions

1. **If I am not going to process my samples immediately, how should I store my samples?**
 - We recommend the use of **Norgen's Urine Preservative** when collecting urine samples, which is designed for the preservation of nucleic acids and proteins in fresh urine samples at ambient temperatures. Urine samples in the preservative should be stored at room temperature. Turbidity or precipitation may be observed if the urine samples are stored at either 4°C or at -20°C. **DO NOT** discard this precipitate and/or spin down your samples to get rid of the turbidity; this will significantly reduce your DNA yields. Make sure to mix your samples thoroughly before processing.
2. **What if a variable speed centrifuge is not available?**
 - A fixed speed centrifuge can be used, however reduced yields may be observed.
3. **What will happen if my centrifugation speed varied from the recommended speed?**
 - This may lead to the degradation of the genomic DNA or reduction in the total DNA yields.
4. **At what temperature should I centrifuge my samples?**
 - All centrifugation steps are performed at room temperature. Centrifugation at 4 °C will not adversely affect kit performance.
5. **Can I process a different urine volume?**
 - Yes, you can process different urine volumes ranging from 3 mL and up to 25 mL without changing the volumes of the buffers outlined in the detailed procedure.
6. **What if I added more or less of the specified reagents' volume?**
 - Adding less volume may reduce your DNA yields. Adding more may not affect the DNA yields EXCEPT if more Elution Buffer was added. Eluting DNA in higher volumes of Elution Buffer will result in diluting your DNA.
7. **What if my incubation time varied from the 20 minutes specified in the product manual?**
 - Less than 20 minutes will result in lower bacterial DNA yields. More than 20 minutes may not affect your bacterial DNA yields but may lower human DNA yields.
8. **What if I forgot to do a dry spin after my third wash?**
 - Your DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with your downstream applications.
9. **Can I elute in a lower volume than indicated?**
 - Yes, you can. The minimum volume you can elute your DNA in is 50 μ L. After eluting your DNA in 50 μ L, reload the eluted DNA back to the column and perform a second elution using the same elution. The final volume of the elution is 50 μ L that has been passed through the column twice.
10. **Why do my samples show very low DNA yield?**
 - 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 could be increased.

11. Why does my DNA not perform well in downstream applications?

- 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.

12. What is the expected DNA yield from urine?

- Urinary DNA yields vary sample to sample. Generally the DNA yield ranges between 50 ng – 2 µg/mL of urine sample. In many cases, DNA yields from urine are too low to be visualized on an agarose gel; however, the DNA yield is sufficient for most downstream applications including PCR and Southern hybridization.

13. I am noticing a white precipitate in my elution. What should I do?

- This white precipitate may appear in the elution depending on the nature of the sample. This white precipitate will not affect your downstream application. Simply mix your elution well before using.

14. Can I process frozen urine sample?

- Frozen samples can be processed. You may notice some precipitation after thawing the urine sample. DO NOT discard any precipitates by either centrifugation or filtration. After thawing mix the urine sample very well before processing.

| Related Products | Product # |
|--|-----------|
| Urine Collection and Preservation Tubes (50 cc) – 1 tube | 18111 |
| Urine Collection and Preservation Tubes (50 cc) – 50 tubes | 18113 |
| Urine Collection and Preservation Tubes (15 cc) – 1 tube | 18120 |
| Urine Collection and Preservation Tubes (15 cc) – 50 tubes | 18122 |
| Urine Collection and Preservation Tubes (5 cc) – 1 tube | 18116 |
| Urine Collection and Preservation Tubes (5 cc) – 50 tubes | 18118 |
| Urine Preservative Single Dose – 1 tube | 18124 |
| Urine Preservative Single Dose – 50 tubes | 18126 |

Product Use Restriction

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The respective user is liable for any and all damages resulting from application of Norgen's Urine DNA Isolation Kit Dx (Slurry Format) for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption.

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 support@norgenbiotek.com.

Authorized Representative



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