

Sputum DNA Isolation Kit

Product # 46200

Product Insert

Norgen's Sputum DNA Isolation Kit provides a rapid procedure for the isolation of sputum DNA. A sputum specimen is the name given to the mucus which is expectorated from the lower airways. The best sputum samples will contain very little saliva, as this contaminates the sample with oral bacteria. Sputum samples are typically evaluated to look for infections such as *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. Other pathogens can also be detected in sputum including HIV. In addition, sputum DNA can be evaluated for the detection of lung cancer or to evaluate chronic inflammation.

INTENDED USE

Norgen's Sputum DNA Isolation Kit constitutes an all-in-one system for the isolation of DNA from sputum samples. The kit allows for the isolation of bacterial or eukaryotic DNA from the sputum samples using spin-column chromatography based on Norgen's proprietary resin. The genomic DNA is isolated free from inhibitors, and can then be used as the template in a number of downstream assays. The kit is designed to allow for the isolation of sputum DNA from 25 samples.

Kit Components:

Component	Contents
Slurry D	55 mL
Proteinase K in Storage Buffer	0.6 mL
Solution WN	4 mL
Wash Solution BE	9 mL
Elution Buffer B	8 mL
Mini Filter Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25

Customer-Supplied Reagents and Equipment

- Disposable powder-free gloves
- Centrifuge with a swinging bucket rotor capable of 2000 RPM.
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- Lysozyme solution (20 mg/mL)
- Dithiothreitol (100 µg/mL) or other solution for upstream sputum homogenization
- 96 – 100% ethanol
- 55°C incubator
- 15 mL conical tubes

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.

Norgen's Sputum DNA Isolation Kit contains ready-to-use Proteinase K, which is dissolved in a specially prepared storage buffer. The buffered Proteinase K is stable for up to 2 years after the date of shipment when stored at room temperature.

General Precautions

All biological samples should be considered as potentially infectious. Proper biosafety measures should therefore be carried out when using this kit.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Sputum DNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Sputum DNA Isolation Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Slurry D**, **Solution WN** and **Wash Solution BE** 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 this solution.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

1. Specimen Collection and Sample Storage

- Expecterated or induced sputum samples may be collected
- It is highly recommended that sputum samples be collected using Norgen's Saliva DNA Collection and Preservation Devices (Cat# RU49000). The sputum samples can be stored for at least two years at room temperature when collected directly using Norgen's Saliva DNA Collection and Preservation Devices.
- Alternatively, sputum samples collected using any other collection and preservation systems or reagents are also compatible with this kit.

2. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogenic material.

B. Isolation of DNA from Sputum

Notes:

- Do not spin down or filter the sputum sample before proceeding with the isolation, as this could negatively affect the isolation of DNA.
- 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 **Proteinase K** solution before use.
- Preheat an incubator or heating block to 60°C.
- Prepare a working concentration of **Slurry D**, **Solution WN** and **Wash Solution BE** by adding the appropriate volume of 96-100% ethanol to the supplied bottles containing the concentrated solutions (see **Table 1** below). The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.
- Prior to the DNA isolation it is recommended that the viscous sputum sample be liquefied. Norgen's Sputum Liquefaction Buffer (Cat. 28289) is recommended. Alternatively, this can be accomplished by adding a reducing agent such as dithiothreitol to the sample and heating at 37°C for 20 minutes to completely homogenize the sample. We recommend preparing a solution of DTT at a concentration of 100 µg/mL and then adding an equal volume to the sputum sample to give a final concentration of 50 µg/mL.
- Up to 1 mL of sputum can be used in the isolation protocol.

Table 1. Volume of Ethanol to be added to Slurry D, Solution WN and Wash Solution BE

Solution	Volume provided	Ethanol (96-100%) Volume to be added by user	Final Volume
Slurry D	55 mL	55 mL	110 mL
Solution WN	4 mL	4 mL	8 mL
Wash Solution BE	9 mL	21 mL	30 mL

1. Add 4 mL of **Slurry D** for every 1 mL of sputum sample in a 15 mL conical tube. Mix well by inversion. (**Note: Slurry D contains silicon carbide resin and must be mixed well before every pipeting.**)
2. Centrifuge for **5 minutes at 2,000 RPM**. Discard the supernatant.
3. Add 20 µL of both **Proteinase K** and **Lysozyme** (user supplied) to the precipitated slurry pellet resulting from the sputum sample. **Vortex for 10 seconds**.
4. Incubate the mixture at **60°C for 20 minutes**.
5. After the 20-minute incubation, add 260 µL **Solution WN**, and mix well by pipetting up and down.
6. Transfer the entire contents into a Mini Filter Spin column (provided) with collection tube.
7. Centrifuge for **1 minute at 14,000 RPM**, and discard the flow-through.
8. Apply 500 µL of **Wash Solution BE** to the column and centrifuge for **1 minute**. Discard the flowthrough and reassemble the spin column with its collection tube.
9. Apply 500 µL of **Wash Solution BE** to the column and centrifuge for **1 minute**. Discard the flow-through and reassemble the spin column with its collection tube.
10. Apply 500 µL of **96-100% Ethanol** (user supplied) to the column and centrifuge for **1 minute**. Discard the flow-through and reassemble the spin column with its collection tube.
11. Spin the column for 2 minutes 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 µL of **Elution Buffer B** to the column and centrifuge for **2 minutes at 2,000 RPM**, followed by **1 minute at 14,000 RPM**.

Frequently Asked Questions

1. What type of container should I use to collect my sputum sample?

Sputum should be collected into a sterile sputum collection cup, vial or tube.

2. What should I do if some of the grey resin is transferred out of the 15 mL conical tube when I am decanting the sputum supernatant?

Simply remix and recentrifuge. After centrifuging decant the supernatant.

3. What if I added more or less of the specified reagents volume?

Adding less volume may reduce DNA yields. Adding more may not affect the DNA yields except when performing the DNA elution step. Eluting DNA in higher volumes of elution buffer will result in diluting your DNA.

4. Can I perform a second elution?

Yes, you can. A second elution is possible, but it is recommended that this elution be performed in a smaller volume (50 µL).

5. Why do my samples show very low DNA yield?

Some sputum samples contain very little DNA. This will vary from individual to individual based on a number of variables. In order to increase the DNA yield it may be necessary to isolate DNA from a larger volume of sputum sample or isolate from several samples and then pool the DNA eluted from the two samples.

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

If a different elution buffer was used from the one provided in the kit, the buffer should be checked for components that may interfere with the application. Common components known to interfere with downstream assays include high salts, EDTA, detergents and other denaturants. Check the compatibility of your elution buffer with its intended use.

7. What if my elution was contaminated with the precipitated resin?

Let the elution stand vertically for 10 minutes to precipitate the resin and then use the clear elution supernatant for downstream applications. This will neither decrease the DNA yield nor interfere with any downstream application.

Related Products	Product #
Saliva DNA Collection and Preservation Devices	RU49000
Norgen's Sputum Liquification Buffer	28289
Bacterial DNA Isolation Kit	17900
Genomic DNA Isolation Kit	24700

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Sputum DNA Isolation Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please 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 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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