

**Sputum DNA Collection, Preservation and Isolation Kit – 50 Individual Devices**  
**Product Insert** **Product #: 46100**

**Norgen’s Sputum DNA Collection, Preservation and Isolation Kit provides a simple and ready to use system for the collection, preservation and isolation of sputum DNA at ambient temperatures.**

**INTENDED USE**

The Sputum DNA Collection, Preservation and Isolation Kit is a complete system for: (1) simple and non-invasive collection of sputum samples; (2) preservation of sputum DNA in sample at ambient temperature for storage and/or shipping and; (3) isolation of high quality DNA within a laboratory setting. The kit contains 50 individual Sputum Collection and Preservation Devices, along with the required reagents for the subsequent isolation of sputum DNA from the preserved samples. Each of the 50 individual kits consists of three components: (1) Sputum Collection Tube; (2) Collection Funnel and; (3) Norgen’s Sputum DNA Preservative contained within sealed single dose ampoules. Fresh sputum samples (either induced or non-induced) are collected by the patient by first rinsing the mouth thoroughly with water. Following this step the patient must then cough deeply to loosen the sputum and then expectorate/spit inside the Collection Funnel which has been assembled with the Collection Tube. Following collection of the sputum sample, the Collection Funnel is removed and the contents of the Preservative ampoule are then added and mixed with the collected sputum and the Collection Tube is labelled. The Sputum Collection Tube is subsequently sent to the laboratory for DNA isolation and analyses. The sputum DNA in preserved samples is stable for more than two years at ambient temperature. The kit is ideal for collecting, preserving and isolating sputum DNA for numerous research based applications, as well as epidemiological and population studies.

**SPUTUM DNA PRESERVATIVE**

Norgen’s Sputum DNA Preservative is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of DNA from fresh specimens. The buffer prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing the resulting samples to be handled and shipped in a safer manner. In addition, the buffer eliminates the need to immediately process or freeze samples and allows the samples to be shipped to centralized testing facilities at ambient temperature. The components of the buffer allow samples to be stored for more than 2 years without any detectable DNA degradation.

**NUCLEIC ACID ISOLATION FROM PRESERVATIVE**

Sputum DNA is isolated from the preserved saliva samples via ethanol precipitation using the provided reagents. The Sputum DNA Preservative is also compatible with other DNA isolation methods, and samples stored in the Sputum DNA Preservative have been used successfully with Norgen’s Sputum DNA Isolation Kit (Cat# 46200).

**Sputum DNA Collection, Preservation and Isolation Kit Contents:**

Component	Contents
Individual Sputum DNA Collection and Preservation Device	50
Sputum Liquefier	2 bottles
Proteinase K	1 vial
Purification Additive	1 vial
Product Insert	1

**Individual Sputum DNA Collection and Preservation Device Contents:**

Component	Contents
Collection Tube	1
Collection Funnel	1
Preservative Ampoule	1
Absorbent Pad	1
Post-Collection Label	1
Donor Instructions	1

Kit Specifications	
Volume of Sputum Collected <sup>a</sup>	2 mL
Volume of Sputum-Preservative Mix	4 mL
Preservation Temperature Range	-20°C to 50°C
Preservation Time	Over 2 years at room temperature
Time to Complete DNA Isolation	2 hours
Average Yield from 0.5 mL*	20 µg
Average OD 260/280	1.7

<sup>a</sup>Volume of sputum collected will vary, but should not exceed 2 mL

\* Average DNA yield will vary depending on the health status of the donor

## MATERIALS REQUIRED BUT NOT SUPPLIED

### 1. For Sample Collection and Preservation

- Scissors

### 2. For DNA Isolation

- Benchtop microcentrifuge
- Micropipettors
- 2 mL microcentrifuge tubes
- 55°C incubator
- 95 - 100% ethanol
- 70% ethanol
- Isopropanol
- TE Buffer (10 mM Tris pH 8.0, 1 mM EDTA)

## SHELF LIFE AND HANDLING

This kit contains ready-to-use Proteinase K which is dissolved in a specially prepared storage buffer. The Proteinase K should be stored at 4°C upon arrival (stable for 2 years) or at -20°C for long term storage (stable for 3 years with minimum freeze and thaw). All other kit components should be kept tightly sealed and stored at room temperature (15 – 25 °C) for up to 3 years without any reduction in kit performance. The Collection Tube, the Collection Funnel and the Device Container of each Individual Sputum DNA Collection and Preservation Device are recyclable.

## QUALITY CONTROL

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

## WARNINGS AND PRECAUTIONS – Sputum Donor

1. If any of the Sputum DNA Preservative comes into contact with skin or eyes, wash thoroughly with water. Do not ingest the Sputum DNA Preservative.

## WARNINGS AND PRECAUTIONS – DNA Isolation from Preserved Sputum Samples

1. Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
2. Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the procedures.
3. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
4. Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
5. As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
6. Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.
7. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

## **Procedures**

### **A. SAMPLE COLLECTION AND PRESERVATION PROCEDURES**

1. Rinse mouth thoroughly with water prior to specimen collection
2. Cough deeply to loosen sputum
3. Expectorate/spit inside **Collection Funnel** assembled with **Collection Tube**
4. Discard/Recycle Collection Funnel
5. Cut the tip off of the **Preservative Ampoule** with scissors
6. Squeeze the Preservative Ampoule to empty the contents into the Collection Tube. Screw the cap onto the Collection Tube to close it tightly
7. Shake the Collection Tube well for 10 seconds to mix the sputum sample and preservative
8. Fill out required information on the provided **Post-Collection Label** and affix it to the Collection Tube. Note: Each of Norgen's Collection Tubes is labeled with a unique serial number that can be used for secure and anonymous tracking of the sample. If required the contents of the Post-Collection Label can be customized to each lab/clinic. For patient privacy a unique identifier instead of the patient's name is recommended
9. The preserved sputum sample can now be stored, shipped or processed immediately
10. Dispose of the Preservation Ampoule and tip in the garbage. Rinse scissors under water and wipe dry
11. The Device container can be disposed of or recycled

### **B. INSTRUCTIONS FOR STORAGE OF PRESERVED SPUTUM SAMPLES**

1. Preserved sputum can be stored at room temperature (20-25°C) for more than 2 years without any significant loss of DNA quality
2. DNA has also been successfully isolated from samples stored at 55°C for 12 months
3. Storage is always recommended at -80°C for archival samples and will provide an optimal preservation. The preservative will freeze at -80°C. Samples stored at -80°C will store indefinitely.

### **C. ISOLATION OF SPUTUM DNA FROM 0.5 mL OF PRESERVED SPUTUM SAMPLES**

**Note:** This procedure outlines the isolation of DNA from 0.5 mL of the preserved sputum samples. Please note that procedures for the isolation of DNA from 4 mL of preserved sputum samples, and using high throughput 96-well formats are available on Norgen's website at [www.norgenbiotek.com](http://www.norgenbiotek.com)

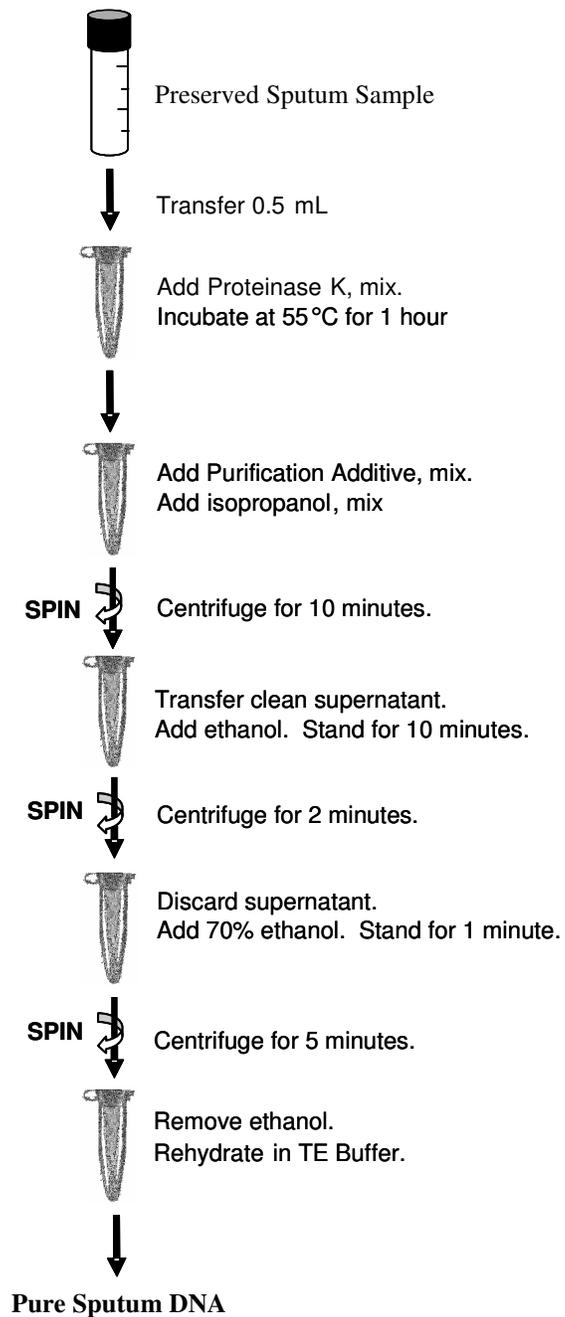
1. Add 4 mL of Sputum Liquefier to the **Collection Tube** and mix the preserved sputum sample in the tube by inversion and gentle shaking for a few seconds. To completely homogenize the sample, place the sample at 37°C for 20 minutes.
2. Transfer 0.5 mL of preserved sputum sample to a 2 mL centrifuge tube (not provided).
3. Add 10 µL of Proteinase K, mix by vortexing for a few seconds and incubate the sample at 55°C for 1 hour.
4. Add 6 µL of Purification Additive to each tube and mix by vortexing for a few seconds.
5. Add 60 µL of isopropanol and mix by vortexing for a few seconds.
6. Centrifuge at room temperature for 10 minutes at maximum speed (14,000 xg).
7. Carefully transfer all the clean supernatant to a new 2 mL centrifuge tube. Discard the pellet.
8. Add an equal volume of room temperature 95-100% ethanol to the clear supernatant. Mix gently by inversion 10 times.
9. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.
10. Centrifuge at room temperature for 2 minutes at maximum speed (14,000 xg).
11. Carefully remove the supernatant, taking care not to disturb the DNA pellet.
12. Carefully add 500 µL of 70% ethanol over the surface of the DNA pellet. Gently swirl and let stand at room temperature for 1 minute.
13. Centrifuge at room temperature for 5 minutes at maximum speed (14,000 x g), then completely remove the ethanol without disturbing the pellet.
14. Keep the tube open and incubate at 65°C for up to 30 minutes to completely dry the DNA pellet. Ensure complete dryness before proceeding to the rehydration step.
15. Rehydrate the DNA pellet in 100 µL of TE Buffer (not provided).
16. Ensure complete rehydration of the DNA (pellet and smear on the side of the tube) prior to any subsequent step. This can be facilitated by incubating the isolated DNA sample at 55°C for 1 hour.

17. The purified DNA sample may be stored at 4 °C up to 2 months. It is recommended that samples be placed at –20 °C for long term storage.

**Note:** Large amounts of high molecular DNA can be very slow to rehydrate (dissolve) completely. Incomplete rehydration of the DNA is a cause of inaccuracy in estimating DNA concentration and failure of downstream applications such as PCR.

### Flowchart

Procedure for Isolating Sputum DNA from Preserved Sputum Samples



Related Products	Product #
Sputum DNA Isolation Kit	46200
Saliva DNA Collection, Preservation and Isolation Kit	35700
Shipping Accessories – 50 Mailers	38300
Saliva DNA Isolation Kit	21410
Saliva DNA Preservation Buffer	21450, 21451, 21452

### 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. We reserve the right to change, alter, or modify any product to enhance its performance and design.

### 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 Collection, Preservation and Isolation Kits 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](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

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