

Sputum DNA Collection, Preservation and Isolation Kit – 50 Individual Devices
Product Insert **Product #: RU46100**

Norgen’s Sputum DNA Collection, Preservation and Isolation Kit provides a safe and rapid all-in-one procedure for the collection, preservation and isolation of sputum DNA at ambient temperatures.

INTENDED USE

Norgen’s Sputum DNA Collection, Preservation and Isolation Kit is an all-in-one solution designed for (1) simple and non-invasive collection of sputum samples; (2) preservation of DNA in sputum samples 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 Sputum Collection and Preservation Devices consists of three components: (1) Sputum Collection Funnel and Collection Tube; (2) Collection Tube Cap 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 is 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. The Sputum Collection Tube is subsequently sent to the laboratory for DNA isolation and analyses. Each of Norgen’s Collection Tubes are labelled with a unique serial number that can be used for secure and anonymous tracking of the sample. The sputum DNA in preserved samples is stable for more than two years at ambient temperature. This 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 non-infectious 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 samples via alcohol 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 x 120 mL
Proteinase K	4.5 mL
Product Insert	1

Individual Sputum DNA Collection and Preservation Device Contents:

Component	Contents
Collection Funnel and Collection Tube	1
Collection Tube Cap	1
Preservative Ampoule	1
Donor Instructions	1

Kit Specifications	
Volume of Sputum Collected ^a	Up to 2 mL
Preservation Temperature Range	-20°C to 50°C
Preservation Time	Over 2 years at room temperature
Time to Complete DNA Isolation	2.5 hours (30 minutes hands on)
Average Yield from 0.5 mL *	20 µg
Average OD 260/280	1.7

^aVolume 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

For DNA Isolation

- Benchtop microcentrifuge
- Micropipettors
- 2 mL microcentrifuge tubes
- 56°C incubator
- Isopropanol
- 70% ethanol
- Isopropanol
- 15 mL Conical tube
- TE Buffer (10 mM Tris pH 8.0, 1 mM EDTA)

SHELF LIFE AND HANDLING

- The Collection Device should be kept tightly sealed and stored at room temperature for up to 3 years without any reduction in kit performance (The collection due date is written on the device label).
- Once collected, sputum is stable for more than 2 years when kept tightly sealed and stored at room temperature.
- The kit contains ready-to-use Proteinase K which is dissolved in a specially prepared storage buffer. The Proteinase K should be stored at -20°C up to the expiry date indicated on the label (with minimum freeze and thaw). The Proteinase K is a white turbid solution. A white precipitate is formed upon storage. Make sure to resuspend the white precipitate by vortexing before use.
- Store Sputum Liquefier at 4°C upon arrival
- 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.

DISCLAIMERS AND SATISFACTION GUARANTEE

For research use only. Not for use in diagnostic procedures.

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.

WARNINGS AND PRECAUTIONS – Sputum Donor

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.
1. Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the procedures.
2. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
3. Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
4. As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
5. 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.
6. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

Procedures

A. SAMPLE COLLECTION AND PRESERVATION PROCEDURES

1. Rinse mouth thoroughly with water prior to specimen collection, and wait for 10 minutes.
2. Cough deeply to loosen sputum
3. Expectorate/spit inside **Collection Funnel** assembled with **Collection Tube** to collect not more than 2 mL of sputum.
4. Discard/Recycle Collection Funnel
5. Twist and remove the tip of the **Preservative Ampoule**
6. Squeeze the Preservative Ampoule to empty the entire 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. The sputum sample is now preserved and ready for storage, shipping or processing.

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.

9. Dispose of the Preservation Ampoule and tip in the garbage. The plastic device container can be recycled

B. INSTRUCTIONS FOR STORAGE OF PRESERVED SPUTUM SAMPLES

Preserved sputum samples can be stored at room temperature for more than 2 years without significant loss of DNA quality.

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. To isolate DNA from other amounts of preserved sputum, simply transfer the desired amount of preserved sputum into a suitable tube and add an equal amount of Sputum Liquifier and proceed with the mixing and incubation as outlined in Step 4 and use a proportional Proteinase K volume. For example, to isolate DNA from 4 mL of preserved sputum transfer the preserved sputum to a 15 mL conical tube, add 4 mL of Sputum Liquifier, and proceed with mixing, incubation and then add 80 μ L of Proteinase K.

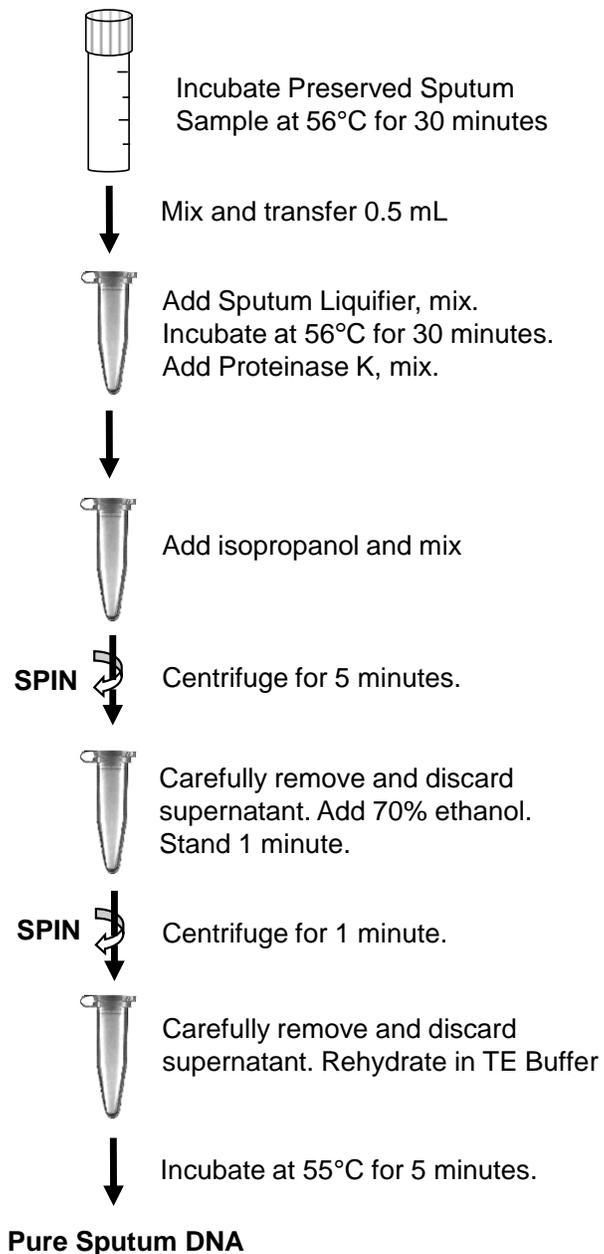
1. Incubate the preserved sputum sample in the Collection Tube at 56°C for 30 minutes in a water incubator or 1 hour in an air incubator.
2. Mix the preserved sputum sample by inversion and gentle shaking or vortexing for a few seconds. Make sure that the sample is as homogenous as possible.
3. Transfer 0.5 mL of preserved sputum to a 2 mL centrifuge tube (not provided).
4. Add 0.5 mL of Sputum Liquifier to the tube, and mix with the preserved sputum sample by inversion and gentle shaking for a few seconds. To completely homogenize the sample, place the sample at 56°C for 30 minutes.
5. Add 10 μ L of Proteinase K (vortex Proteinase K before use), mix by vortexing for a few seconds and incubate the sample at 56°C for 1 hour.
6. Add an equal volume of room temperature isopropanol to the sample. Mix gently by inversion 10 times.
7. Centrifuge at room temperature for 5 minutes at maximum speed (14,000 xg).
8. Carefully remove and discard the supernatant, taking care not to disturb the DNA pellet. Gently place the tube briefly upside down on a paper towel to remove residual isopropanol.
9. Carefully add 500 μ L of 70% ethanol. Gently swirl and let stand at room temperature for 1 minute.
10. Centrifuge at room temperature for 1 minute at maximum speed (14,000 x g), then carefully remove and discard the ethanol without disturbing the pellet.
11. Gently place the open tube upside down on paper towel for 10-15 minutes to remove the excess amount of 70% EtOH and to air dry the DNA pellet.
12. Add 100 μ L of TE Buffer (not provided). Vortex for 30 seconds and incubate at 55°C for 5 minutes to rehydrate the DNA pellet. Ensure complete rehydration of the DNA (pellet and smear on the side of the tube) prior to any subsequent step.

Note:

- a. If a higher DNA concentration is required for downstream applications, the DNA pellet can be rehydrated in 50 μ L of TE buffer.
 - b. Large amounts of high molecular DNA can be very slow to rehydrate (dissolve) completely. Incomplete rehydration of the DNA will affect estimating the DNA concentration and can result in failure of downstream applications such as SNP, digestion and PCR.
13. Centrifuge the tube at 14,000 x g for 1 minute to pellet any insoluble material.
 14. Transfer the clear liquid into a clean tube taking care not to disturb the pellet.
 15. 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.

Flowchart

Procedure for Isolating Sputum DNA from Preserved Sputum Samples



Related Products	Product Number
Sputum DNA Isolation Kit	46200
Saliva DNA Collection, Preservation and Isolation Kit	RU35700
Shipping Accessories – 50 Mailers	38300
Saliva DNA Isolation Kit	RU45400

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) or through email at techsupport@norgenbiotek.com.

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