Saliva DNA Isolation Reagent Kit (up to 4 mL)  
Product #: RU35720

INTENDED USE
Norgen’s Saliva DNA Isolation Reagent Kit (up to 4 mL) is designed for the isolation of high quality DNA from up to 4 mL of preserved saliva samples within a laboratory setting. The kit contains sufficient reagents to perform 50 DNA isolations from up to 4 mL of preserved saliva samples collected using Norgen’s Saliva DNA Collection and Preservation Devices, or via other preservation methods.

Saliva DNA Isolation Reagent Kit (up to 4 mL) Contents:

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase K</td>
<td>8 mL</td>
</tr>
<tr>
<td>Binding Buffer B</td>
<td>85 mL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

MATERIALS REQUIRED BUT NOT SUPPLIED
- Benchtop swing bucket centrifuge (capable 3000 x g to 5000 x g)
- 55°C incubator
- Micropipettors
- 2 mL microcentrifuge tubes
- 15 mL centrifuge tube
- 70% ethanol
- Isopropanol
- TE Buffer (10 mM Tris pH 8.0, 1 mM EDTA)

SHELF LIFE AND HANDLING
- 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 for up to 3 years. The Proteinase K is a white turbid solution. A white precipitate is formed upon storage. **Make sure to re-suspend the white precipitate by vortexing before use.**
- Binding Buffer B should be kept tightly sealed and stored at room temperature. It can be stored for up to 3 years.

QUALITY CONTROL
In accordance with Norgen’s Quality Management System, each lot of Norgen’s Saliva DNA Isolation Reagent Kit (up to 4 mL) 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 – DNA Isolation from Preserved Saliva Samples

1. Follow universal precautions. All 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 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.

Flowchart

Procedure for Isolating Saliva DNA from 4 mL of Preserved Saliva Samples.
To process different saliva input volumes please see the table under Section B.

- Mix preserved saliva sample and transfer 4 mL
- Add Proteinase K, mix.
  - Incubate at 55°C for 15 minutes.
  - Add Binding Buffer B, mix.
  - Incubate at 55°C for 5 minutes
- Add isopropanol and mix
- Centrifuge for 10 minutes.
- Carefully remove and discard supernatant. Add 70% ethanol.
  - Stand 1 minute.
- Centrifuge for 5 minutes.
- Carefully remove and discard ethanol. Rehydrate in TE Buffer.
- Centrifuge and transfer.

Pure Saliva DNA
B. ISOLATION OF SALIVA DNA FROM 4 mL OF PRESERVED SALIVA SAMPLES

Note: To process different saliva input volumes, please refer the table below for required reagent amounts.

<table>
<thead>
<tr>
<th>Input Volume (preserved saliva)</th>
<th>Proteinase K</th>
<th>Binding Buffer B</th>
<th>Isopropanol (equal volume)</th>
<th>70% Ethanol</th>
<th>TE Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mL</td>
<td>40 µL</td>
<td>400 µL</td>
<td>1440 µL (1.44 mL)</td>
<td>1mL</td>
<td>200 µL</td>
</tr>
<tr>
<td>2 mL</td>
<td>80 µL</td>
<td>800 µL</td>
<td>2880 µL (2.88 mL)</td>
<td>1mL</td>
<td>300 µL</td>
</tr>
<tr>
<td>3 mL</td>
<td>120 µL</td>
<td>1200 µL (1.2mL)</td>
<td>4320 µL (4.32 mL)</td>
<td>1mL</td>
<td>400 µL</td>
</tr>
<tr>
<td>4 mL</td>
<td>150 µL</td>
<td>1600 µL (1.6mL)</td>
<td>5750 µL (5.75 mL)</td>
<td>1mL</td>
<td>500 µL</td>
</tr>
</tbody>
</table>

1. Mix the preserved saliva sample by inversion and gentle shaking for a few seconds.

2. Transfer 4 mL of preserved saliva sample to a 15 mL centrifuge tube (not provided).

3. Add 150 µL of **Proteinase K** (make sure to vortex proteinase K before use), mix by vortexing for 10 seconds and incubate the sample at 55°C for 15 minutes.

4. Add 1.6 mL of **Binding Buffer B** to the sample. Mix by vortexing for 10 seconds and incubate the sample at 55°C for 5 minutes.

5. Add an equal volume (total volume of preserved saliva, Proteinase K and Binding Buffer B) of room temperature **isopropanol** to the sample. Mix gently by inversion 10 times.

6. Centrifuge at room temperature for 10 minutes at a minimum of 3,500 x g (~ 4,000 RPM).

7. 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.

8. Carefully add 1 mL of 70% ethanol. Gently swirl and let stand at room temperature for 1 minute.

9. Centrifuge at room temperature for 5 minutes at a minimum of 3,500 x g (~ 4,000 RPM), then carefully remove and discard the 70% ethanol without disturbing the pellet.

10. Gently place the open tube upside down on paper towel for 10 minutes to remove the excess amount of 70% EtOH and to air dry the DNA pellet.

11. Rehydrate the DNA pellet in 500 µL of TE Buffer (not provided). Pipette or vortex to dissolve the pellet. Ensure complete rehydration of the DNA (pellet and smear on the side of the tube) prior to any subsequent step. Incubate at 55 °C for 30 minutes with occasional vortexing

**Note:**
- If a higher DNA concentration is required for downstream applications, DNA pellet can be rehydrated in 250 µL of TE buffer.
- 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.

12. Transfer all the liquid to a 1.5 mL microcentrifuge tube and centrifuge at 20,000 x g (~14,000 RPM) for 1 minute to pellet any insoluble material.

13. Transfer the clear liquid into a clean tube taking care not to disturb the pellet.

14. 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.
### Related Products

<table>
<thead>
<tr>
<th>Product Description</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva DNA Collection, Preservation and Isolation Kit – 50 Individual Devices</td>
<td>RU35700</td>
</tr>
<tr>
<td>Saliva DNA Isolation Kit</td>
<td>RU45400</td>
</tr>
<tr>
<td>Saliva DNA Collection and Preservation Devices (50)</td>
<td>RU49000</td>
</tr>
<tr>
<td>Shipping Accessories – 50 Mailers</td>
<td>38300</td>
</tr>
</tbody>
</table>

### 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 Saliva 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 distributers ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at techsupport@norgenbiotek.com.