Supplementary Protocol for DNA Isolation from Saliva Preserved with Norgen's Saliva DNA Collection and Preservation Device using Qiagen QIAamp DNA Blood Mini Kit

Components Required

<table>
<thead>
<tr>
<th>Component</th>
<th>Product #</th>
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<tbody>
<tr>
<td>Saliva DNA Collection and Preservation Device</td>
<td>35710*</td>
</tr>
<tr>
<td>QIAamp DNA Blood Mini Kit</td>
<td>Qiagen 51104 (50 preps)</td>
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<td></td>
<td>Qiagen 51106 (250 preps)</td>
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</tbody>
</table>

* Part of Norgen’s Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700) and Norgen’s Saliva DNA Collection and Preservation Devices (50) (Cat# 49000)

Storage Conditions and Product Stability (For Norgen’s Product)
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, saliva is stable for more than 2 years when kept tightly sealed and stored at room temperature.

Storage Conditions and Product Stability (For Qiagen’s Product)
Please refer to manufacturer’s documentation for storage condition and product stability.

Precautions and Disclaimers
Saliva 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 saliva. If any of the Saliva DNA Preservative comes into contact with skin or eyes, wash thoroughly with water. Do not ingest the Saliva DNA Preservative. The collection device contains a choking hazard (Collection Tube Cap). Keep away from children.

Customer-Supplied Reagents and Equipment
- Incubator at 55°C
- Pipettes with aerosol barrier tips
- 96 – 100 % Ethanol

Notes Prior to Use
- Ensure that all solutions are at room temperature prior to use.
- The protocol below describes the isolation of saliva DNA from the recommended input of 200 µL of preserved saliva sample
- The maximum input amount of preserved saliva mixture is 400 µL per spin column. Please refer to the various notes in Section 1 (Saliva Sample Collection and Lysate Preparation) for the appropriate amount of solutions to be added

1. Saliva Sample Collection and Lysate Preparation
   a. Collect and preserve saliva samples using Norgen’s Saliva DNA Collection and Preservation Device (#35710), according to the enclosed instruction.
   b. Before using the preserved saliva for DNA isolation, mix the preserved saliva by inversion for a few seconds
   c. Incubate the preserved saliva at 55°C for a minimum of 1 hour.
      **Note:** The incubation could be performed anytime after the collection but before the DNA extraction. **If the saliva is to be used immediately after collection, incubate the preserved saliva for a minimum of 2 hours.**
   d. After the incubation, mix the preserved saliva by inversion for a few seconds
   e. Aliquot 200 µL of preserved saliva (already incubated at 55°C) to a microcentrifuge tube (not provided).
f. Aliquot 20 µL of Protease to the microcentrifuge tube containing the preserved saliva.

**Note**: For input volumes greater than 200 µL of preserved saliva, use 10 µL of Protease for every 100 µL of preserved saliva.

g. Add 200 µL Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.

**Note**: For input volume greater than 200 µL of preserved saliva, use 100 µL of Buffer AL for every 100 µL of preserved saliva.

h. Incubate at 55°C for 10 minutes.

i. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.

j. Add 200 µL ethanol (96 – 100%) to the sample, and mix again by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.

**Note**: For input volume greater than 200 µL of preserved saliva, use 100 µL of ethanol (96 – 100%) for every 100 µL of preserved saliva.

2. **Column Purification of Saliva DNA**

a. Carefully apply the mixture from step 1j to the QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 minute. Discard the flowthrough and reassemble the spin column with the collection tube.

b. Repeat step 2a until all lysate has passed through the spin column. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided), and discard the collection tube containing the filtrate.

c. Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 minute. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided), and discard the collection tube containing the filtrate.

d. Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 minutes.

e. Recommended: Place the QIAamp Mini spin column in a new 2 mL collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.

f. Place the QIAamp Mini spin column in a clean 1.5 mL microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µL Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 minute, and then centrifuge at 6000 x g (8000 rpm) for 1 minute.

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**Related Products**

<table>
<thead>
<tr>
<th>Related Products</th>
<th>Product #</th>
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<tbody>
<tr>
<td>Saliva DNA Isolation Kit</td>
<td>45400</td>
</tr>
<tr>
<td>Saliva DNA Collection, Preservation and Isolation Kit</td>
<td>35700</td>
</tr>
<tr>
<td>Saliva DNA Collection and Preservation Devices (50)</td>
<td>49000</td>
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</tbody>
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**Technical Support**
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. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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