

Olive Oil DNA Isolation Kit

Product # 61700

Product Insert

Norgen's **Olive Oil DNA Isolation Kit** is designed for the rapid preparation of genomic DNA from olive oil in order to support genetic traceability by allowing for the verification of the authenticity of the oil, thereby discouraging adulteration. Norgen's Olive Oil DNA Isolation Kit can also be used to purify DNA from other processed oils such as canola oil, sunflower oil or vegetable oil to allow for downstream analysis and testing for oil adulteration. Genomic DNA is efficiently extracted from 0.5 mL of oil by a combination of heat treatment, homogenization and a unique lysis buffer. PCR inhibitors are effectively removed during the washing steps and the purified genomic DNA is ready to use for downstream applications such as PCR and microarray analysis. Preparation time for ten samples is less than 30 minutes, and each kit contains sufficient materials for 50 preparations.

Norgen's Purification Technology

Purification is based on spin column chromatography. The process involves first adding Lysis Buffer L to the 0.5 mL oil. Next, the mixture is homogenized and then incubated at 65°C for 10 minutes. The sample is then centrifuged, and the supernatant is transferred to a DNase-free microcentrifuge tube. Ethanol and Binding Buffer B are added to the lysate, and it is then loaded onto one of the provided spin columns. Norgen's columns bind nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the proteins are removed in the flowthrough or retained on top of the resin. The bound DNA is then washed twice using the provided Wash Solution A, and the purified DNA is eluted using the Elution Buffer. The purified DNA can be used in sensitive downstream applications including PCR.

Kit Specification

| Kit Specifications | |
|-----------------------------------|------------|
| Maximum Column Binding Capacity | 10 µg |
| Maximum Column Loading Volume | 650 µL |
| Time to Complete 10 Purifications | 25 minutes |

Kit Components

| Component | Product # 61700 (50 preps) |
|------------------------|----------------------------|
| Lysis Buffer L | 60 mL |
| Binding Buffer B | 40 mL |
| Wash Solution A | 38 mL |
| Elution Buffer B | 8 mL |
| Homogenization Tubes | 50 |
| Spin Columns | 50 |
| Collection Tubes | 50 |
| Elution tubes (1.7 mL) | 50 |
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Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 2 year in their unopened containers

Advantages

- Fast and easy processing using rapid spin-column format
- Isolate total DNA without compromising total yield
- No phenol or chloroform extractions
- Isolate high quality total DNA from a variety of oil samples

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. 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.

The **Binding Buffer B** contains guanidine salts, and should be handled with care. Guanidine salt forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- A flat-bed vortex pad with tape, or any commercially available bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 Instrument).
- 2 mL microcentrifuge tubes
- 65°C water bath or heating block
- 96 – 100% ethanol

Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

where RCF = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g -force.

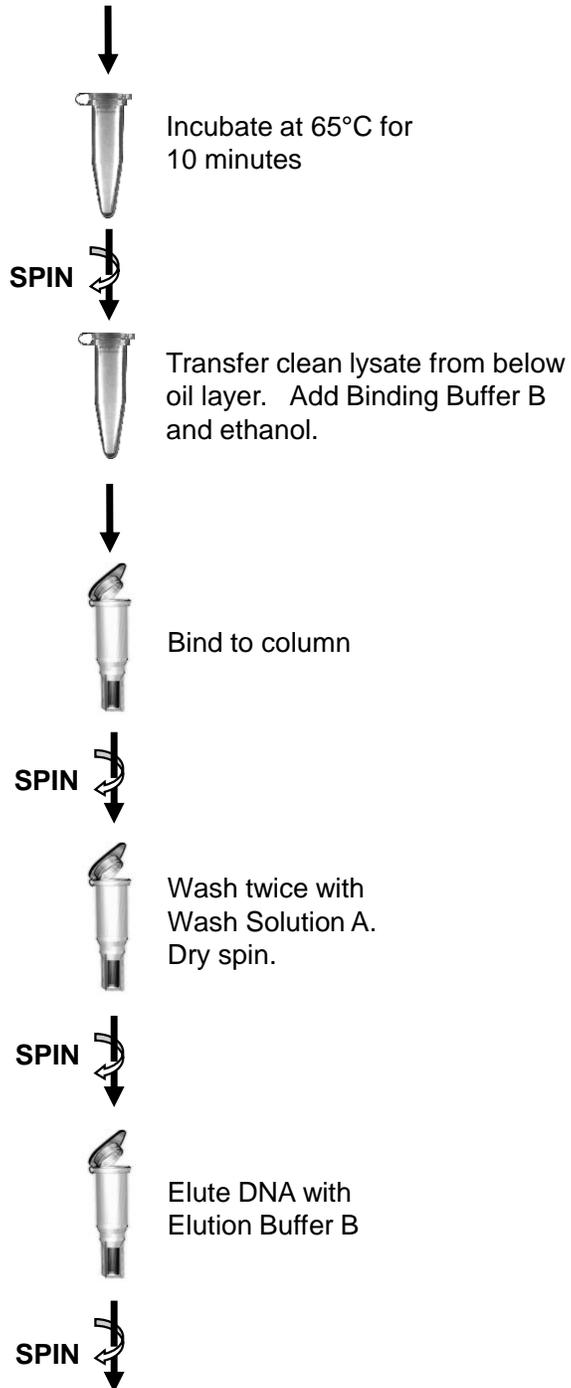
Notes prior to use:

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- 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.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle(s) containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Preheat a water bath or heating block to 65°C.

Flowchart

Procedure for Isolating Total DNA using Norgen's Olive Oil DNA Isolation Kit

Add oil to Homogenization Tube. Add 1 mL of **Lysis Buffer L**. Vortex.



Purified Total DNA

1. Lysate Preparation

- a. Transfer 0.5 mL of olive oil or other processed oil to a provided homogenization tube.
- b. Add 1 mL of **Lysis Buffer L**.
- c. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 Instrument). Vortex for 60 seconds at 4M/S using a FastPrep®-24 instrument, or 5 minutes using a flat-bed vortexer at maximum speed. Alternatively, optimize the time and speed according to the manufacturer's manual.
- d. Incubate the tube at 65°C for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
- e. Centrifuge the tube for 1 minute at **20,000 × g (~14,000 RPM)**.
- f. Clean lysate will be separated from oil layer. Carefully take up to 650 µL of the clean lysate under the oil layer by pipetting and transfer to a 2 mL DNase-free microcentrifuge tube (provided by the user) without disturbing the oil layer.

Note: Trace amounts of oil carried over to the lysate should not affect the quality of DNA, however too much oil in the lysate may affect DNA yield. In that case, repeat Step 1d and 1e to get a clean lysate.

- g. Add 650 µL of **Binding Buffer B** to the lysate collected above. Vortex to mix.
- h. Add 650 µL of **96%-100% ethanol** (provided by the user) to the lysate above. Vortex to mix.
- i. **Proceed to Step 2: Binding to Column.**

2. Binding to Column

- a. Assemble a column with one of the provided collection tubes
- b. Apply up to 650 µL of the lysate with **ethanol** and **Binding Buffer B** onto the column and centrifuge for 1 minute at **10,000 × g (~10,000 RPM)**. Discard the flowthrough and reassemble the spin column with the collection tube.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **20,000 × g (~14,000 RPM)**.

- c. Repeat Step **2b** with remaining lysate.

3. Column Wash

- a. Apply 600 µL of **Wash Solution A** to the column and centrifuge for 1 minute at **10,000 × g (~10,000 RPM)**.

Note: Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute at **20,000 × g (~14,000 RPM)**.

- b. Discard the flowthrough and reassemble the column with its collection tube.
- c. Repeat step **3a** to wash column a second time.
- d. Discard the flowthrough and reassemble the spin column with its collection tube.
- e. Spin the column for 2 minutes at **20,000 × g (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 40 μL of **Elution Buffer B** to the column.
- c. Incubate the column at room temperature for 2 minutes.
- d. Centrifuge for 30 seconds at **2,000 x g (~2,000 RPM)** followed by 1 minute at **20,000 x g (~14,000 RPM)**.

5. Storage of DNA

The purified DNA may be stored at -20°C for a few weeks. It is recommended that samples be placed at -70°C for long term storage.

Troubleshooting Guide

| Problem | Possible Cause | Solution and Explanation |
|--|---|--|
| The spin column is clogged. | Improper oil removal | Too much oil was carried over to the lysate. Repeat Step 1d and 1e to minimize the oil carry over. Clogging can be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column. |
| DNA does not perform well in downstream applications | Low DNA yield | Depending on the oil extraction process DNA can be damaged or lost. Increase the input volume up to 1mL, as well as the other buffers and solutions proportionally. |
| | Ethanol carryover | Ensure that the dry spin under the Column Wash procedure is performed in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications. |
| | The column was not washed twice with the provided Wash Solution A | Traces of salt from the binding step may remain in the sample if the column is not washed twice with the Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column. |
| | PCR reaction conditions need to be optimized | Take steps to optimize the PCR conditions being used, including varying the amount of template (20 ng to 50 ng for 20 μL of PCR reaction), changing the source of <i>Taq</i> polymerase, adding BSA (final concentration is 0.1 $\mu\text{g}/\mu\text{l}$), looking into the primer design and adjusting the annealing conditions. |

| Related Products | Product # |
|---------------------------------------|------------------|
| Microm DNA isolation | 55300 |
| Water RNA/DNA Purification Kit | 26400 |
| Fungi/Yeast Genomic DNA Isolation Kit | 27300 |
| Plant/Fungi DNA Isolation Kit | 26200 |

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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