NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Plant/Fungi DNA Isolation 96 Well Kit — Product Insert

Product # 26900

Norgen's Plant/Fungi DNA Isolation 96-Well Kit provides a rapid method for the high-throughput isolation of total DNA from a wide range of plant and fungi species. Furthermore, the kit also provides a convenient method for the high throughput detection of pathogens which may be infecting a plant, as it allows for the co-purification of any pathogen DNA including bacterial and yeast, along with the purification of the total DNA. Total DNA can be purified from fresh or frozen plant tissues, plant cells or fungi samples using this kit. The DNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform. The purified DNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, Southern blotting, RFLP and SNP assays.

Norgen's Purification Technology

Purification is based on 96-well column chromatography. The DNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The purification could be performed on either a vacuum manifold or using centrifugation. The process involves grinding plant tissue in a mortar with liquid Nitrogen (or alternative homogenization equipment). Lysis Buffer L and RNase A are then added, followed by a short incubation at 65°C. Next, Binding Buffer I is then added to the lysate followed by another short incubation on ice. The lysate is then spun in order to pellet and remove any debris. Ethanol is then added to the lysate, and the solution is loaded onto the 96-Well Plate. The 96-well plate binds DNA in a manner that depends on ionic concentrations. Thus only the DNA will bind to the plate while the contaminating proteins will be removed in the flowthrough. The bound DNA is then washed with the provided Solution WN and Wash Solution A in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. The purified DNA is of the highest integrity, and can be used in a number of downstream applications.

Specifications

Kit Specifications	
Binding Capacity Per Well	50 µg
Maximum Loading Volume Per Well	500 µL
Size of DNA Purified	All sizes
Maximum Amount of Starting Material: Fungi Plant Tissues	 50 mg (wet weight) 50 mg
Time to Complete 96 Purifications	50 minutes
Average Yields* Grape leaf Apple leaf Strawberry leaf Plum leaf Peach leaf Peach petiole	 7 µg 3 µg 6 µg 4 µg 5 µg 4 µg

***** *average yields will vary depending upon a number of factors including species, growth conditions used and developmental stage.*

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature, except for the RNase A which should be stored at -20°C. This kit is stable for 1 year after the date of shipment.

Advantages

- Fast and easy high throughput processing using either a vacuum manifold or centrifugation
- Isolate total DNA from a variety of plant and fungal species, including any pathogen DNA
- No phenol or chloroform extractions
- Isolate high quality total DNA from a variety of sources

Kit Components

Component	Product # 26900 (192 preps)
Lysis Buffer L	2 x 60 mL
Binding Buffer I	25 mL
Solution WN	55 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	30 mL
RNase A	1 vial
96-Well Plate	2
96-Well Collection Plate	2
Adhesive Tape	4
96-Well Elution Plate	2
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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*.

Solution WN contains guanidinium salts and should be handled with care. Guanidinium salts 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

You must have the following in order to use the Plant/Fungi DNA Isolation 96-Well Kit:

- Micropipettors and multichannel pipettes
- 70% and 96%-100% ethanol
- 65°C Incubator
- Ice bath
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (96-well format) for centrifugation
- Cell Disruption Tool such as mortar and pestle, rotor-stator homogenizer or bead mills
- For ***Vacuum Format***:
    - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
    - Sealing tape or pads
- For ***Centrifuge Format***:
    - Centrifuge with rotor for 96-well plate assembly (Eppendorf 5810 / 5810 R or Eppendorf 5910 / 5910 R)

Flowchart

Procedure for Purifying Total DNA using Norgen's Total DNA Purification 96-Well Kit

Grind plant or fungi using liquid nitrogen.
Add Lysis Buffer L and RNase A

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Incubate at 65°.
Add Binding Buffer I.
Incubate on ice.

↓

Add ethanol

Bind DNA

↓

Wash once with Solution WN.
Wash twice with Wash Solution A.

↓

Elute DNA with
Elution Buffer B

↓

Purified Total DNA

Procedures

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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

- The isolation of DNA from plants and fungi can be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B. Lysate preparation is the same for both procedures.
- The lysate can either be prepared in microcentrifuge tubes or in a 96-well plate. Both methods for lysate preparation are described below.
- Ensure that all solutions are at room temperature prior to use. If necessary, warm to 65°C to redissolve any precipitates. Prepare a working concentration of **Solution WN** by adding 73 mL of 96 - 100% ethanol (to be provided by the user) to the supplied bottle containing concentrated Wash Solution A. This will give a final volume of 128 mL. The labels on the bottles have a box that can be checked to indicate that ethanol has been added.
- 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 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.
- The volumes stated in each procedure for lysate preparation are the volumes required to prepare samples for each well of the 96-well plate.
- Store the RNase A solution at 4 °C for up to 3 months. For longer storage, the RNase A solution should be divided into small aliquots and stored at -20 °C.
- The maximum recommended input of plant tissue and fungi (wet weight) is 50 mg or per well of the 96-Well Plate.

1. Lysate Preparation

a. Place ≤50 mg of plant tissue or wet fungi into a mortar that contains liquid Nitrogen and grind into a powder. Add 400 µL of **Lysis Buffer L** and 1 uL of **RNase A** (provided) to the plant or fungi powder and transfer the entire lysate mixture to 1.7 mL microcentrifuge tube. Alternatively, other homogenization methods can be used with this procedure, including a bead system. If an alternative method is used, add 400 µL of **Lysis Buffer L** to the sample immediately after homogenization and vortex for 20 seconds to mix.
b. Incubate the tube or plate at 65ºC for 10 minutes. Add 80 µL of **Binding Buffer I** and incubate on ice for 5 minutes.
c. After incubation spin the tubes for 5 minutes at 14,000 x g, or the 96-well plate for 5 minutes at maximum speed or 4,000 *x g* (~4,000 RPM), to pellet any cell debris.

d. Using a pipette, transfer the clean lysate into a new DNAase-free microcentrifuge tube (not provided).
e. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Total DNA Isolation

Note: The purification of total DNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in 2A. For purification using centrifugation, please follow the procedure outlined in 2B

A. Total DNA isolation Using Vacuum Manifold

2. Binding DNA to 96-Well Plate

a. Assemble the 96-Well Plate and the vacuum manifold according to manufacturer's recommendations.

Note: The provided 96-Well Collection Plate can be used as the collection/waste tray if desired.

b. Apply up to 500 μL of the lysate mixed with the ethanol (from **Step 1**) into each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application of the mixture to the wells.

c. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Plate and the vacuum manifold.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

3. DNA Wash

a. Apply 500 μL of **Solution WN** to each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the 96-Well Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Plate and the vacuum manifold.
d. Apply 500 μL of **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells and apply vacuum for 2 minutes.
e. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
f. Reassemble the 96-Well Plate and the vacuum manifold. Repeat steps **3d** and **3e** to wash column for a third time. Pat the bottom of the 96-Well Plate dry. Reassemble the

96-Well Plate and the vacuum manifold. Apply vacuum for an additional 5 minutes in order to completely dry the plate.

b. Turn off vacuum and ventilate the manifold.

4. DNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided Elution Plate. Complete the vacuum manifold assembly with the 96-Well Plate.
b. Add 75 µL of **Elution Buffer B** to each well of the plate.
c. Apply vacuum for 2 minutes.

5. Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Total DNA Purification Using Centrifugation

Note: To purify total DNA using a vacuum manifold please follow Section A above.

2. Binding DNA to 96-Well Plate

a. Place the 96-Well Plate on top of a provided 96-Well Collection Plate
b. Apply up to 500 µL of the lysate mixed with the ethanol (from **Step 1**) into each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 2 minutes.

Note: Depending on the starting material, a small quantity of precipitates may appear in the lysate-ethanol mix. No additional step is required to remove these precipitates prior to application to the wells

c. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.

Note: Ensure that all of the lysate from each well has passed through into the bottom plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. DNA Wash

a. Apply 500 µL of **Solution WN** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the bottom plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate.
c. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Plate. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 RPM) for 2 minutes.
d. Discard the flowthrough. Reassemble the 96-Well Plate and the bottom plate
e. Repeat steps **3c** and **3d** to wash column for a third time.
f. Gently pat the bottom of the 96-Well plate on a paper towel to remove residual wash buffer.

g. Reassemble the 96-Well Plate and the bottom plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 10 minutes in order to completely dry the plate.

4. DNA Elution

a. Stack the 96-Well Plate on top of the Elution Plate.
b. Add 75 -100 µL of **Elution Buffer B** to each well of the 96-Well Plate and incubate for 1 minute at room temperature.
c. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 3 minutes.

5. Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Buffer L was used for the amount of cells or tissue.
	Wells of the plate have become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the wells of the plate show clogging below the recommended levels. See also “Clogged Wells in Plate”
	An alternative elution solution was used	It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery.
	70% Ethanol was not added to the clean lysate	Ensure that the appropriate amount of 70% ethanol is added to the lysate before binding to the wells of the plate.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
	Low DNA content in cells or tissues used	Different tissues and cells have different DNA contents, and thus the expected yield of DNA will vary greatly from these different sources. Please check literature to determine the expected DNA content of your starting material.

Problem	Possible Cause	Solution and Explanation
Clogged Wells in Plate	Insufficient Vacuum	Ensure that a vacuum pressure of at least -650 mbar or -25 in. Hg is developed
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the wells to clog.
DNA does not perform well in downstream applications	PCR reaction conditions need to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of *Taq* polymerase, looking into the primer design and adjusting the annealing conditions.
	DNA was not washed 2 times with the provided Solution WN and Solution A	Traces of salt from the binding step may remain in the sample if the plate is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry vacuum or dry spin under the DNA Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.

Related Products	Product #
Plant/Fungi RNA Purification kit	25800
Plant RNA/DNA Purification kit	24400
Plant/Fungi DNA Isolation kit	26200
100b RNA Ladder	15002
1kb RNA Ladder	15003

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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