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Direct Fungi DNA Isolation Kit — Product Insert

Product # 25600

Norgen's Direct Fungi DNA Isolation kit provides a rapid method for the isolation and purification of genomic DNA from the fungi found on the surface of symptomatic plant samples. The major benefit of this kit is that it does not require the use of physical homogenization steps to isolate the fungal DNA, which are often time consuming and tedious procedures. While a mortar and pestle, a rotor-stator homogenizer or bead mills associated with liquid nitrogen may enhance the DNA extraction yield, they are not suitable methods for use in the analysis of large numbers of samples. And in the area of pathogen diagnosis, simple and convenient sample preparation steps are a critical factor in supporting high throughput analysis. Therefore, Norgen's Direct Fungi DNA Isolation Kit adopts a simple and rapid sample preparation procedure that does not rely on physical homogenization steps to isolate high quality fungal DNA that can be used in sensitive downstream detection methods. The procedure yields sufficient fungal DNA to be used in a number of downstream applications including real time PCR, sequencing, Southern blotting and SNP analysis. The total procedure can be completed less than 60 minutes.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The process involves first collecting the berries or plant tissue of interest and shaking them with a small volume of Collection Solution in order to extract the surface fungi. Next, the Collection Solution is collected and centrifuged, and the supernatant is removed from the pellet. Resuspension Solution and Lyticase are then added to the pellet, and the mixture is incubated at 37°C for 30 minutes. Lysis Solution is then added to the mixture, and again it is incubated at 65°C for 30 minutes. The lysate is then centrifuged to remove any cell debris, and an equivalent volume of ethanol is added to the lysate. Next, the mixture is loaded onto a spin-column. Norgen's resin will bind nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the proteins and degraded RNA are removed in the flowthrough. The bound DNA is then washed twice times with the provided Wash Solutions in order to remove any impurities, and the purified fungal DNA is eluted with the Elution Buffer.

Specifications

Kit Specifications	
Column Binding Capacity	15 µg
Maximum Column Loading Volume	600 µL
Maximum Amount of Starting Material: Symptomatic Grapes Plant Tissue	 1 – 2 berries (<1 gram total weight) <1 gram total weight
Time to Complete 10 Purifications	60 minutes

Kit Components

Component	Product # 25600 (25 samples)
Lysis Solution	15 mL
Resuspension Solution	8 mL
Wash Solution I	15 mL
Wash Solution II	7.5 mL
Elution Buffer	8 mL
Mini Spin Columns	25
Collection Tubes	25
Elution tubes (1.7 mL)	25
Sample Collection Bag	25
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Advantages

- Rapid isolation and purification of fungal DNA from the surface of infected plants
- No liquid nitrogen or physical disruption methods required
- Fast and easy processing using rapid spin-column format
- Isolate high quality genomic DNA
- No phenol or chloroform extraction

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 1 year in their unopened containers.

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*.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Direct Fungi DNA Isolation Kit:

- Benchtop microcenrifuge
- 2 mL microcentrifuge tubes
- 37ºC and 65ºC water bath or heating block
- 96-100% ethanol
- 70% ethanol
- Lyticase
- β-mercaptoethanol
- Sorbitol
- 0.9 % NaCl

Flow Chart

Procedure for Purifying Total Fungal DNA using Norgen's
Direct Fungi DNA Isolation Kit

Shake plant sample with Collection Solution. Transfer solution to centrifuge tube.

Purified Total Fungi DNA

Procedures

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

- All centrifugation steps are carried out in a benchtop microcentrifuge at **14,000 x g (~ 14,000 RPM)** except where noted. All centrifugation steps are performed at room temperature.
- 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.
- Prepare a working concentration of the **Wash Solution II** by adding 7.5 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. This will give a final volume of 15 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare the **Collection Solution** by making a 0.9% NaCl solution (provided by the user). Two milliliters of **Collection Solution** are required for each preparation. A larger volume may be prepared and stored at room temperature.
- Add 10 μL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Solution** required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Add 1μL of β-mercaptoethanol (provided by the user) and 180 μg of Sorbitol to each 1 mL of **Resuspension Solution** required and mix well. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- Pre-heat two separate water baths or heating blocks to 37ºC and 65ºC.

1. Lysate Preparation

a. Place 1 or 2 symptomatic grape berries or a small sample of plant tissue (< 1 g total weight) into the provided **Sample Collection Bag**.
b. Add 2 mL of **Collection Solution** (provided by the user) to the bag and seal tightly.
c. Shake the samples gently in the tube or bag in order to extract the exterior fungi into the **Collection Solution.**

   Note: It is important to ensure that the skin of the berry is not broken during the shaking. If the skin does break, new berry samples should be obtained and the protocol should be repeated from Step **1a**.

d. Using a pipette, transfer the liquid to 2 mL microcentrifuge tube (provided by the user) and centrifuge at **14000 × g (~14,000 RPM)** for 1 minute.
e. Using a pipette remove the supernatant carefully so as not disturb or dislodge the cell pellet.

f. Add 250 μL of **Resuspension Solution** and 200 units of lyticase (provided by the user) to the cell pellet and vortex to resuspend.
g. Incubate at 37ºC for 30 minutes.
h. Add 500 μL of **Lysis Solution** to the solution and mix by vortexing.
i. Incubate at 65ºC for 15 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
j. Centrifuge at **14000 × g (~14,000 RPM)** for 1 minute to separate the supernatant.
k. Transfer the supernatant (approximately 750 μL) into a new 1.7 mL microcentrifuge tube using a pipette, and add 275 μL of 96-100% ethanol to the supernatant and mix well.
l. 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 600 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **14000 × g (~14,000 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.

c. Depending on your lysate volume, repeat step **2b** if necessary.

3. Column Wash

a. Apply 500 μL of **Wash Solution I** to the column and centrifuge at **14000 × g (~14,000 RPM)** for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the column with the collection tube.
c. Apply 500 μL of **Wash Solution II** to the column and centrifuge at **14000 × g (~14,000 RPM)** for 1 minute.
d. Discard the flowthrough and reassemble the column with the collection tube.
e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. Elution of Clean DNA

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of **Elution Buffer** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **14,000 x g (~14,000 RPM)** for 1 additional minute.
d. **(Optional):** Additional elution may be performed if desired. Another 100μl of **Elution Buffer** may be added to the column and repeat step **4c**. The total yield can be improved by additional 20-30% when this second elution is performed.

5. Storage of DNA

The purified nucleic acids 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
Clogged Column	Too much cell debris in the lysate supernatant	Ensure that most cell debris is removed in Step **1d**, when transferring the supernatant to a new microcentrifuge tube.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.
DNA does not perform well in downstream applications	Column was not washed two times with the provided Wash Solutions	Traces of salt from the binding step may remain in the sample if the column is not washed with the provided Wash Solutions. Salt may interfere with downstream applications, and thus must be washed from the column.
	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.
Yield of Genomic DNA is low	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Lysis Solution was used, and ensure that the lysate was incubated at 65°C for 15 minutes. If necessary, incubate the lysis solution for an extra 5 minutes to assist in lysis.
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 *x g* for 1 minute is performed following the 2 minute centrifugation at 200 *x g.* Also, ensure that the entire volume of Elution Buffer passed through the columns and is eluted.
Genomic DNA is Sheared	Sample is old	Ensure that the sample is not too old, as old samples often yield only degraded DNA
	Sample repeatedly frozen and thawed	Samples should not be repeatedly frozen and thawed, as this tends to increase the likelihood of isolating degraded DNA.

Related Products	Product #
Plant RNA/DNA Purification Kit	24400
Yeast Genomic DNA isolation kit	18600
1kb RNA Ladder	15003
UltraRanger 1kb DNA Ladder	12100

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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