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Fungi/Yeast Genomic DNA Isolation 96-Well Kit Product Insert

Product # 27350

Norgen's Fungi/Yeast Genomic DNA Isolation 96-Well Kit provides a fast, reliable and simple procedure for high throughput isolation of DNA from viable yeast cells, fungal spores or mycelium and Gram-positive bacteria. Genomic DNA is efficiently extracted from the cells by a combination of heat treatment, detergents and the use of provided Bead Tubes. The purified DNA is of the highest quality and is fully compatible with any downstream applications such as real-time PCR and sequencing. The option of an additional lyticase treatment is also provided in order to allow for improved DNA yields for certain fungal and yeast species.

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 first adding Lysis Buffer L to pelleted cells (or fungi, yeast or Gram positive bacteria), and transferring the cells to a provided Bead Tube to homogenize the sample. Optional lyticase treatment can be done at this point. The sample is then incubated at 65°C for 10 minutes and then centrifuged, and the supernatant is transferred to a DNase-Free microcentrifuge tube. An equal volume of 96-100% ethanol and Solution BX are then added to the lysate. Next, the solution is loaded onto a 96-Well Plate, which binds only the DNA. The bound DNA is then washed using the provided Wash Solution A, and the purified DNA is eluted using the Elution Buffer B. The purified total DNA is free of all inhibitors and can be used in a number of downstream applications, including PCR and sequencing.

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 (wet weight) Yeast or gram positive bacterial culture	 50 mg 0.5 mL -1 mL
Time to Complete 96 Purifications	40 minutes

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Advantages

- Fast and easy high throughput processing using either a vacuum manifold or centrifugation.
- Rapid high throughput method to isolate genomic DNA from different fungi, yeast and Gram-positive bacteria.
- No phenol or chloroform extractions.
- High yield and quality DNA that is ready for PCR and other downstream applications.

Kit Components

Component	Product # 27350 (192 preps)
Lysis Buffer L	2 x 60 mL
Resuspension Solution A	60 mL
Solution BX	2 x 28 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	30 mL
Bead Tubes (Bag of 25)	8
96-Well Plate	2
96-Well Collection Plate	2
Adhesive Tape	4
96-Well Elution Plate	2
Product Insert	1

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for 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 BX contains guanidinium salts and should be handled with care. Guanidinium salts form 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 Fungi/Yeast DNA Isolation 96-Well Kit:

- Micropipettors and multichannel pipettes
- 96-100% ethanol
- Water bath or heating block for 65°C
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (96-well format) for centrifugation
- DNase-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 instrument)
- For ***Vacuum Format***:
    - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -60 kpa or -15 in. Hg (such as PALL Life Sciences Multi-Well Plate Vaccum manifold)
    - Sealing tape or pads
- For ***Centrifuge Format***:
    - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)

Flowchart

Procedure for Purifying Total DNA using Norgen's Fungi/Yeast Genomic DNA isolation 96 well kit

Add sample and Lysis Buffer L to Bead Tube

↓ Vortex for 5 minutes. Incubate at 65°C for 10 minutes. Centrifuge. Transfer lysate.

Add ethanol.
Add Solution BX.

↓ Transfer lysate.

Bind to 96-Well Plate

↓ **SPIN**

Wash two times with
Wash Solution A

↓ **SPIN**

Elute DNA with
Elution Buffer B

↓ **SPIN**

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

- 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 each of the supplied bottles containing the concentrated **Wash Solution**. 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 maximum yeast or bacteria input amount should not exceed 1 x 10^8 cfu's. Depending on culture growth, this is equivalent to approximately 0.5 to 1.0 mL of an overnight culture.
- For the isolation of genomic DNA from fungal species other than yeast, **Collection Solution** must be prepared. **Collection Solution** consists of 0.9% (w/v) NaCl prepared with distilled water.
- Preheat a water bath or heating block to 65°C.
- **Optional:** The DNA yield from some fungal or yeast species may be increased by performing an optional **Lyticase lysis step**. If desired, prepare a Lyticase stock solution according to the supplier's instruction. It is recommended that the stock solution have a minimum concentration of 4 units per μL. Aliquot and store the unused portions at -20°C until needed.

Section 1. Lysate Preparation

A. Lysate Preparation (Fungi Growing on Plates or Culture)

a. **Fungi Growing on Plates:** Add approximately 5 mL (volume can be adjusted based on density of fungal growth) of **Collection Solution** (see notes before use) to the plate and gently collect fungal spores and mycelium with an inoculation loop or autoclaved pipette tip, ensuring not to collect any agar debris. Transfer up to 1 mL of washed spores and wet mycelium to a microcentrifuge tube (provided by user).
Fungi in Culture: For fungi growing in a culture, transfer 50 mg of fungi (wet weight) to a microcentrifuge tube.
b. Centrifuge at 14,000 x g (~14,000 RPM) for 1 minute to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

c. Add 500 μL of **Lysis Buffer L** to the cell pellet. Resuspend the cells by gentle vortexing.

(Optional RNase A treatment) If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of RNase A (not to exceed 20 μL) to the cell suspension.

d. Transfer the mixture to a provided Bead Tube and secure the tube horizontally on a flat-bed vortex pad with tape, or in any commercially available bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 instrument).

e. Vortex for 5 minutes at maximum speed on a flat-bed vortexer or optimize the condition for any commercially available bead beater equipment.

Note: Foaming during the homogenization is common. This foaming is due to detergents present in the Lysis Buffer L and will not affect the protocol.

f. Incubate the **Bead Tube** with lysate at 65°C for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
g. Briefly spin the tube to remove liquid from the cap, and transfer all of the lysate, including cell debris, to a DNase-free microcentrifuge tube (provided by the user) by pipetting. Ensure that the beads are not transferred during the pipetting.
h. Centrifuge the tube for 2 minutes at **14000 × g (~14,000 RPM)**.
i. Carefully transfer clean supernatant to a new DNase-free microcentrifuge tube (provided by the user) without disturbing the pellet. Note the volume. Alternatively a 96 deep well plate-2.0 mL (provided by the user) can be used. Caution must be taken to avoid well to well cross contamination.
j. Add an equal volume of **96-100% 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. If using a 96 well plate, mix gently by pipetting (5 times up and down).
k. Add 300 μL of **Solution BX** and briefly vortex to mix. If using a 96 well plate, mix gently by pipetting (5 times up and down).
l. **Proceed to Step 2:** Binding DNA to 96-Well Filter Plate.

B. Lysate Preparation (Yeast and Gram-positive Bacteria)

a. Transfer up to 1 mL of yeast or Gram-positive bacterial culture (maximum input 1 x 10^8 cfu's) to a microcentrifuge tube and centrifuge at 14,000 x g (~14,000 RPM) for 1 minute to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.
b. **Proceed to Step Ac above.**

C. Lysate Preparation with Lyticase (Optional)

In general, no extra enzymes are required to lyse fungal and yeast cells when using this kit. However, the combination of using the Bead Tubes with Lyticase will improve DNA yield for certain fungal, yeast and Gram-positive bacterial species. If low DNA yield is expected, please follow the alternative lysis step provided below.

a. Follow step **Aa and Ab** as above.
b. Add 250 μL of **Resuspension Solution A** to the cell pellet. Resuspend the cells by gentle vortexing.
c. Add 200 units of Lyticase (see **Notes prior to use**) and mix well. Incubate at 37°C for 45 minutes.

Note: The time for incubation may vary from 30 minutes to 1 hour. Please refer to the Lyticase manufacturer's instruction.

d. Proceed to step **Ac above.**

Section 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 A2. For purification using centrifugation, please follow the procedure outlined in B2

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 and Solution BX (from Se 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.

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 **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user). 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 a n additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Repeat step **3a** and **3b**.
d. Reassemble the 96-Well Plate and the vacuum manifold. Apply vacuum for an additional 10 minutes in order to completely dry the plate.
e. Turn off vacuum and ventilate the manifold.
f. Gently pat the bottom of the 96-Well Plate on a clean paper towel to remove residual wash buffer.
g. Replace the collection/waste tray in the vacuum manifold with the provided Elution Plate. Complete the vacuum manifold assembly with the 96-Well Plate.
h. Incubate at 65°C for 5 minutes.

4. DNA Elution

a. Add 100 µL of **Elution Buffer B** to each well of the plate.
b. Apply vacuum for 5 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 F 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 and Solution BX (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: Ensure the entire wash solution has passed through into the collection plate by inspecting the 96-Well Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

3. DNA Wash

a. 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.

Note: Ensure the entire wash solution has passed through into the collection 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 collection plate.

c. Repeat step 3a and 3b.

d. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 10 minutes in order to completely dry the plate.

e. Gently pat the bottom of the 96-Well Plate on a clean paper towel to remove residual wash solution.

f. Stack the 96-Well Plate on top of the 96-Well Elution Plate.

g. Incubate at 65°C for 5 minutes

4. DNA Elution

a. Add 100 µL of **Elution Buffer B** to each well of the 96-Well Plate and incubate for 1 minute at room temperature.

b. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 5 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
The 96-Well Plate is clogged.	The sample is too large	Too many cells were applied to the wells of the plate. Ensure that the amount of cells used is less than 1 x 10^8 viable yeast cells or 50 mg (wet weight) of fungal culture. Clogging can be alleviated by increasing the g-force, vacuum pressure and/or centrifuging time for a longer period of time until the lysate passes through the column.
Turbid elution	The sample is too large	Depending on fungi species, sometimes turbidity can be observed in the elution. This may inhibit downstream applications. Reduce the amount of cells used, and perform a third wash during the Wash step.
Poor DNA Recovery	Lysis was not completed	Increase the incubation time at 65ºC to 15 minutes.
	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.
	Solution BX was not added to the lysate	Solution BX enhances DNA binding to the column for maximum DNA recovery.
DNA does not perform well in downstream applications	The sample is too large	Too many cells were applied to the column. Ensure that the amount of cells used is less than 1 x 10^8 viable yeast/bacterial cells or 50 mg (wet weight) of fungal culture.
	Ethanol carryover	Ensure that the dry spin under the Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
	The 96-Well Plate 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 *Taq* polymerase, adding BSA (final concentration is 0.1 μg/μl), looking into the primer design and adjusting the annealing conditions.

Related Products	Product #
Plant/Fungi DNA Isolation Kit	26200, 26900
Fungi/Yeast Genomic DNA Isolation Kit	27300
Bead Tubes	26533, 26534

Technical Support
Contact our Technical Support Team between the hours of 9:00 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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