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Fungi/Yeast RNA/DNA Purification Kit — Product Insert

Product # 35800

Norgen's **Fungi/Yeast RNA/DNA Purification Kit** is designed for the rapid preparation of total RNA and genomic DNA from viable yeast cells and fungi. Total RNA, including siRNA, and genomic DNA are efficiently extracted from the cells by a combination of heat treatment, detergents and the use of provided Bead Tubes. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications such as PCR and Southern Blot analysis. Typical yields of total RNA and genomic DNA will vary depending on the cell density of the yeast or fungal culture and species. Additional lyticase treatment is optional in order to improve RNA and DNA yield for certain fungal and yeast species. Preparation time for a single sample is less then 30 minutes, and each kit contains sufficient materials for 50 preparations.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin. The process involves first adding Lysis Solution to the fungi or yeast sample. At this point, an optional RNase treatment can be performed if RNA-free genomic DNA is required. Next, the mixture is placed into a provided Bead Tube and the tube is vortexed for 5 minutes in order to efficiently and rapidly homogenize the sample and extract the RNA and DNA. The sample is then centrifuged, and the supernatant is transferred to a nuclease-free microcentrifuge tube. Ethanol is added to the lysate, and it is then loaded onto one of the provided spin columns containing Norgen's proprietary resin. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the RNA and DNA will bind to the column while the proteins are removed in the flowthrough or retained on top of the resin. The bound nucleic acids are then washed twice using the provided Wash Solution, and the purified nucleic acids are eluted using the Elution Buffer.

Kit Specification

Kit Specifications	
Maximum Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Maximum Amount of Starting Material: Fungi (wet weight) Yeast culture	 50 mg 0.5 mL-1mL
Average Yields* Yeast DNA Yeast RNA	 5-30 µg 5-30 µg
Time to Complete 10 Purifications	30 minutes

* Yield will vary depending on the type of sample processed

Advantages

- Rapid and convenient method to isolate total RNA and genomic DNA from yeast and fungi samples
- Isolate total RNA, including microRNA and siRNA
- Fast and easy processing using rapid spin-column format
- No phenol or chloroform extractions

Kit Components

Component	Product # 35800 (50 preps)
Lysis Solution	30 mL
Resuspension Buffer	18 mL
Wash Solution	18 mL
Elution Buffer	6 mL
Bead Tubes	50
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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. The Proteinase K should be stored in aliquots at -20°C upon reconstitution. This product is stable at room temperature in its lyophilized form.

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*.

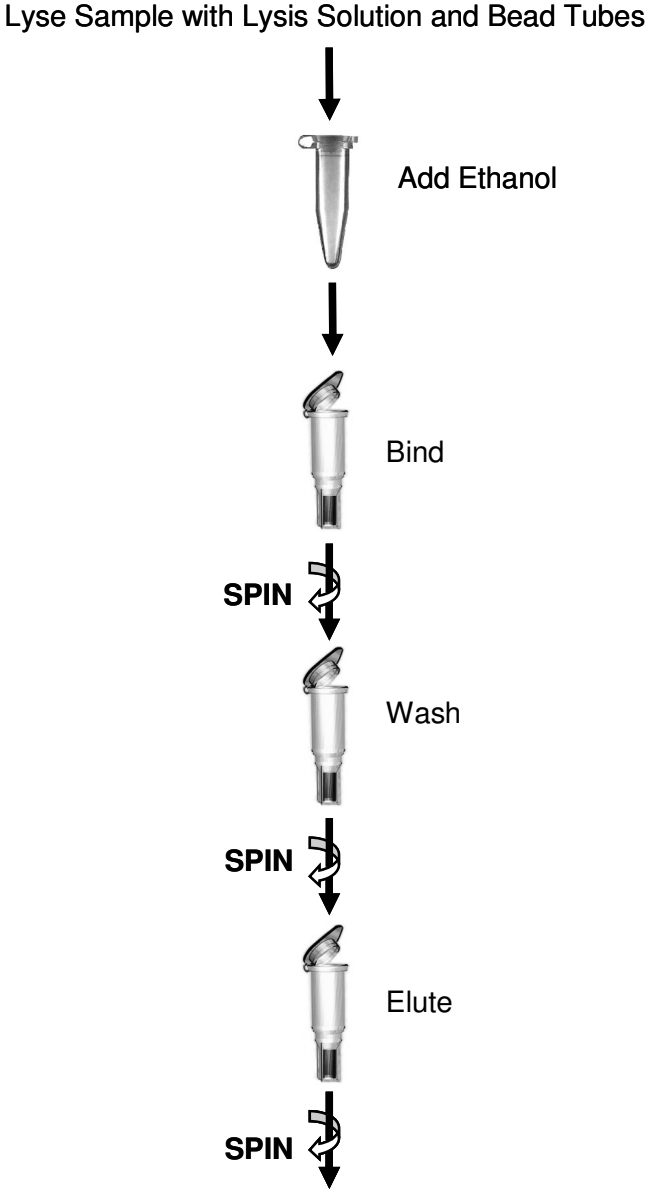
Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 65°C water bath or heating block
- 96 – 100% ethanol
- Lyticase (optional)

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Flowchart

Procedure for Purifying Total RNA and Genomic DNA using Norgen's Fungi/Yeast RNA/DNA Purification Kit



Pure Fungi/Yeast Total RNA and Genomic DNA

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** by adding 42 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The input yeast cell amount should not exceed 1 x 10^8 cfu's. Depending on culture growth, this is equivalent to 0.5 to 1.0 mL of an overnight culture.
- For the isolation of total RNA and genomic DNA from fungal species other than yeast, Collection Solution must be prepared. Collection Solution is 0.9% (w/v) NaCl prepared in distilled water.
- Preheat a water bath or heating block to 65°C.

1A. Lysate Preparation (Yeast)

a. Transfer up to 1 mL of yeast culture 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. Add 500 µL of **Lysis Solution** to the cell pellet. Resuspend the cells by gentle vortexing.
c. Transfer all the mixture to the 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. Scientific Industries' Disruptor Genie™).

   Note: The appearance of some white foam during the homogenization is common. This is due to detergents present in the Lysis Buffer and will not affect the protocol.

d. Vortex for 5 minutes at maximum speed.
e. Incubate at 65°C for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
f. Briefly spin the tube to remove liquid from the cap.
g. Transfer the all lysate including cell debris to a nuclease-free microcentrifuge tube (provided by the user).
h. Centrifuge the tube for 2 minute at **14000 × g (~14,000 RPM)**.
i. Carefully transfer clean supernatant to nuclease-free microcentrifuge tube (provided by the user). Note the volume

j. 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.
k. **Proceed to Step 2: Binding to Column**

1B. Lysate Preparation (Fungi Growing on Plates or Culture)

a. With an inoculation loop, collect fungal colonies on a plate and resuspend in 1 mL of Collection Solution in a microcentrifuge tube. From a culture it is recommended that no more than 50 mg of fungi (wet weight).
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. **Proceed to Step 1A (b).**

1C. Alternative lysis with Lyticase (Optional)

In general, the use of Lyticase is not required to lyse fungal cells in this kit. However the combination of the Bead Tubes and Lyticase will improve RNA and DNA yield for certain fungal species. If low RNA and DNA yield is expected, please follow this alternative lysis step.

Prepare a lyticase stock solution according to the supplier's instruction. It is recommended to have a minimum concentration of 4 units per µL. Aliquot and store the unused portions at -20°C until needed.

a. Follow **Steps 1B (a) and 1B (b).**
b. Add 250 µL of **Resuspension Solution** to the cell pellet. Resuspend the cells by gentle vortexing.
c. Add 200 units of Lyticase (see notes above) 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. Add 500 µL of **Lysis Solution** and resuspend the cells by gentle vortexing.
e. Proceed to **Step 1A (b)**

2. Binding Nucleic Acids to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply up to 600 µL of the lysate with ethanol onto the column and centrifuge for 1 minute at **14,000 x 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** to the column and centrifuge 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 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 in order to thoroughly dry the resin. Discard the collection tube.

4. Nucleic Acid Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 100 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):** An additional elution may be performed if desired by repeating steps **4b** and **4c** using 100 µL of Elution Buffer. The total yield can be improved by an additional 20 – 30% when this second elution is performed.

5. Storage of DNA and RNA

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.

Related Products	**Product #**
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100
Bacterial Genomic DNA Isolation Kit	17900
Blood Genomic DNA Isolation Kit	18200
Saliva DNA Isolation Kit	21410
Fungi/Yeast genomic DNA isolation kit	27300

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.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
The micro spin column is clogged	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 cells or 50 mg (wet weight) of fungi from the culture. 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.
Poor RNA and DNA Recovery	Lysis was not completed	Ensure suggested lysis volume was applied. Alternatively, increase the incubation time at 65ºC to 15 minutes.
	Ethanol was not added to the Wash Solution	Ensure that 70 mL of 95 - 100% ethanol is added to the supplied Wash Solution prior to use.
	An alternative elution buffer was used	It is recommended that the Elution Buffer supplied with this kit be used for maximum RNA and DNA recovery.
RNA and DNA does not perform well in downstream applications	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 Solutions	Traces of salt from the binding step may remain in the sample if the column is not washed twice with the Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	PCR reaction conditions is 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.

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