Geneaid

Instruction Manual
Ver. 08.29.17
For Research Use Only

Presto™ Mini RNA Yeast Advanced Kit

RBYA004, RBYAD004 (4 Preparation Sample Kit)
RBYA050, RBYAD050 (50 Preparation Kit)
RBYA100, RBYAD100 (100 Preparation Kit)
RBY300, RBYD300 (300 Preparation Kit)

Advantages

Sample: a variety of yeast and other fungus species
Yield: up to 50 µg of RNA (5 x 10^7 *Saccharomyces cerevisiae*)
Format: certified DNase and RNase-free beadbeating tubes and spin columns
Time: within 30 minutes
Elution Volume: 50 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The Presto™ Mini RNA Yeast Advanced Kit is designed for rapid isolation of total RNA from cultured yeast and fungus. The yeast/fungus cells are directly added into beadbeating tubes containing ceramic beads and lysis buffer. Samples are efficiently lysed without the use of digestive enzymes (such as Lyticase or Zymolase) using a bead beating instrument or a standard vortex. The cell lysate is then mixed with Binding Buffer and the RNA is bound by the RB column. The column is then washed and the purified total RNA is eluted with RNase-free Water. High quality total RNA can be purified in less than 30 minutes without phenol extraction or alcohol precipitation. The purified RNA is ready for use in RT-PCR, northern blotting, primer extension, mRNA selection and cDNA synthesis.

Quality Control

The quality of the Presto™ Mini RNA Yeast Advanced Kit is tested on a lot-to-lot basis by isolating RNA from *Saccharomyces cerevisiae* (5×10^7) harvested by centrifugation at 5,000 x g for 10 minutes. A 5 µl aliquot of purified RNA from a 50 µl eluate is analyzed by electrophoresis on a 0.8% agarose gel.

Kit Components

Component	RBYA004 RBYAD004	RBYA050 RBYAD050	RBYA100 RBYAD100	RBYA300 RBYAD300
RT Buffer	1.5 ml x 2	40 ml	75 ml	200 ml
PR Buffer	1 ml	6 ml	15 ml	40 ml
RB Buffer	2 ml	30 ml	60 ml	130 ml
DNase I[1] (2U/µl) (RBYAD004/050/100/300 Only)	20 µl	275 µl	550 µl	550 µl x 3
DNase I Reaction Buffer (RBYAD004/050/100/300 Only)	200 µl	2.5 ml	5 ml	15 ml
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1.5 ml (6 ml)	25 ml (100 ml)	25 ml + 12.5 ml (100 ml) (50 ml)	50 ml x 2 (200 ml x 2)
RNase-free Water	1 ml	6 ml	15 ml	30 ml
Beadbeating Tubes Type B	4	50	100	300
RB Columns	4	50	100	300
2 ml Collection Tubes	8	100	200	600

[1]DNase I is shipped at room temperature and should be stored at -20ºC for extended periods after receiving the kit.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Steps to prevent RNase contamination

1. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

2. Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.

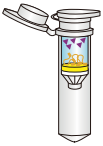
3. Non-disposable glassware or plasticware should also be sterile (RNase-free).

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram

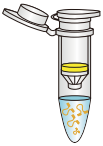
Lysis of yeast and other fungus species using beadbeating tubes and a standard vortex or bead beating instrument.

Enzymatic cell lysis using Lyticase or Zymolase is not required.



RNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while RNA remains bound to membrane)



Elution of pure total RNA which is ready for subsequent reactions

Presto™ Mini RNA Yeast Advanced Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation

2. All centrifuge steps are at room temperature

DNA Removal Options: For DNA-free RNA perform either option 1 (following RNA Binding) or option 2 (following RNA Elution).

Additional Requirements

1.5 ml microcentrifuge tubes, standard vortex, ß-mercaptoethanol or 2M Dithiothreitol, absolute ethanol, EGTA (for DNA Digestion In Solution)

Yeast/Fungus Protocol Procedure

1. Sample Preparation

A. Yeast/Fungus on Agar Plate

Use an inoculating loop to transfer **a small piece of yeast/fungus (up to 5 x 10^7)** from an agar plate to a 1.5 ml microcentrifuge.

B. Yeast/Fungus in Broth

Transfer **yeast/fungus cells** in broth to a 1.5 ml microcentrifuge tube. Centrifuge for 10 minutes at 5,000 x g then discard the supernatant. Weigh **10-50 mg of wet pellet (up to 5 x 10^7)**. Repeat harvesting yeast/fungus cells by centrifugation using the same microcentrifuge tube if required.

2. Cell Lysis

Add **600 µl of RT Buffer and 6 µl ß-mercaptoethanol (or 12 µl of freshly prepared 2M Dithiothreitol in RNase-Free Water) to the spheroplast pellet** then vortex or pipette to re-suspend. Transfer the re-suspended yeast/fungus cells to a **Beadbeating Tube**. Attach the Beadbeating Tubes horizontally on a standard vortex with tape or use an adapter. Vortex at maximum speed for 5 minutes. Carefully open the cap then add **100 µl of PR Buffer**. Vortex briefly to eliminate the foam caused by detergents. Incubate the Beadbeating Tubes on ice for 5 minutes. Centrifuge at 11,000 x g for 3 minutes then transfer **400 µl of supernatant** to a clean 1.5 ml microcentrifuge tube (RNase-free).

3. RNA Binding

Add **400 µl of RB Buffer** and **400 µl of absolute ethanol** to the sample and mix IMMEDIATELY by shaking vigorously for 10 seconds.

NOTE: An equal volume of RB Buffer and absolute ethanol can be mixed in advance and stored at room temperature. Transfer 800 µl of RB Buffer and ethanol mixture to the sample then mix by shaking vigorously.

Place a **RB Column in a 2 ml Collection Tube**. Transfer **600 µl of sample mixture** to the RB Column. Centrifuge at 16,000 x g for 1 minute then discard the flow-through. Place the RB Column back in the 2 ml Collection Tube. Transfer the remaining sample mixture to the RB Column then centrifuge at 16,000 x g for 1 minute. Discard the flow-through then place the RB Column in a new 2 ml Collection Tube.

Optional Step 1: In Column DNase I Digestion

DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield.

1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.

2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.

3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

4. Gently pipette DNase I solution to mix (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.

5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with the RNA Wash step.

4. RNA Wash

Add **400 µl of W1 Buffer to the RB Column** then centrifuge at 16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. **Add 600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 16,000 x g for 30 seconds. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column** again. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Centrifuge at 16,000 x g for 3 minutes to dry the column matrix.

5. RNA Elution

Place the **dried RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). **Add 50 µl of RNase-free Water** into the **CENTER** of the column matrix. Let stand for at least 3 minutes to ensure the **RNase-free Water** is absorbed by the matrix. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Optional Step 2: DNA Digestion In Solution

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2. Gently pipette the DNase I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65ºC for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the Geneaid™ RNA Cleanup Kit instead of stopping the reaction with EGTA.

Presto™ Mini RNA Yeast Advanced Kit Functional Test Data

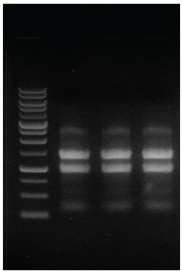


Figure 1. Total RNA was extracted using the Presto™ Mini RNA Yeast Advanced Kit. *Saccharomyces cerevisiae* (5×10^7) was harvested by centrifugation at 5,000 x g for 10 minutes. A 5 µl aliquot of purified RNA from a 50 µl eluate was analyzed by electrophoresis on a 0.8% agarose gel.

M = Geneaid 1 Kb DNA Ladder

Sample	ng/ul	260/280	260/230	Yield (µg)
1	728.3	2.24	2.51	36.4
2	777.5	2.23	2.52	38.9
3	702.6	2.23	2.51	35.1

Troubleshooting

Low Yield

Too much sample was used.

Reduce the amount of starting material or separate it into multiple tubes. The yeast/fungus sample amount should not exceed 5 x10^7 cells.

Sample lysis or homogenization was incomplete.

Horizontally vortex the Beadbeating Tube at maximum speed for 5 minutes. Alternatively, a Disruptor Genie or similar can be used.

Inappropriate buffer preparation.

Add appropriate volume of absolute ethanol (see the bottle label) to Wash Buffer prior to use.

RNA Degradation.

Use fresh cultured cells or cell pellets frozen at -70ºC. Extracted RNA should be stored at -70ºC. Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures. Non-disposable glassware or plasticware should also be sterile (RNase-free).

RNA contaminated with genomic DNA.

Perform In Column DNase I Digestion or DNA Digestion in Solution step to eliminate DNA contamination.

Eluted RNA does not perform well in downstream applications

Residual Ethanol Contamination.

Following the wash step, dry the RB Column with additional centrifugation at 14-16,000 x g for 5 minutes.

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Registration Number 046

CERTIFICATE NO. QAIC/TW/50077

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