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IBI SCIENTIFIC

# INSTRUCTION MANUAL

## rYeast Total RNA Mini Kit

IB47410 (4 Preparation Sample Kit)

IB47411 (100 Preparation Kit)

IB47412 (300 Preparation Kit)

# Advantages

**Sample:** a variety of yeast and other fungus species
**Yield:** up to 30 µg of RNA (5 x $10^7$ Saccharomyces cerevisiae: 20 µg)
**Convenient:** includes Sorbitol Buer to reduce sample preparation time
**Format:** certied DNase and RNase-free spin columns
**Time:** within 20 minutes
**Elution Volume:** 50-100 µl
**Kit Storage:** dry at room temperature (15-25°C)

# Introduction

The rYeast Total RNA Mini Kit was designed for total RNA purification from yeast and a wide variety of other fungus species. Sorbitol Buffer is included with the kit to reduce sample preparation time and minimize hands on time. Detergents and chaotropic salt are used to lyse cells and inactivate RNase while RNA is bound by the glass fiber matrix of the RNA spin column. Once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNase-free Water. High quality total RNA can be purified within 20 minutes and is ready for use in RT-PCR, Northern Blotting, Primer Extension, mRNA Selection and cDNA Synthesis.

# Quality Control

The quality of the rYeast Total RNA Mini Kit is tested on a lot-to-lot basis by isolating RNA from Saccharomyces cerevisiae ($5\times10^7$) 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 Contents

| Component | IB47410 | IB47411 | IB47412 |
|---|---|---|---|
| Sorbitol Buffer | 4.5 ml | 90 ml | 225 ml |
| RB Buffer | 2 ml | 60 ml | 130 ml |
| DNase I1 (2U/µl) | 20 µl | 550 µl | 550 µl x 3 |
| DNase I Reaction Buffer | 200 µl | 5 ml | 15 ml |
| W1 Buffer | 2 ml | 50 ml | 130 ml |
| Wash Buffer2 (Add Ethanol) | 1.5 ml (6 ml) | 25 ml + 12.5 ml (100 ml) (50 ml) | 50 ml x 2 (200 ml) x 2 |
| RNase-free Water | 1 ml | 15 ml | 30 ml |
| RB Columns | 4 | 100 | 300 |
| 2 ml Collection Tubes | 8 | 8 | 600 |

[1] DNase I is shipped at room temperature and stored at -20°C 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.

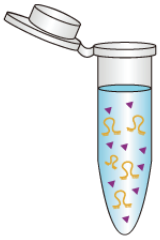
# Steps to prevent RNase contamination

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).

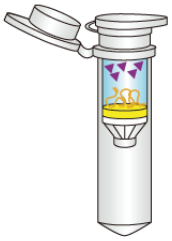
**IMPORTANT BEFORE USE!**

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

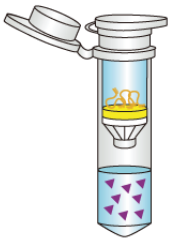
# Quick Protocol Diagram



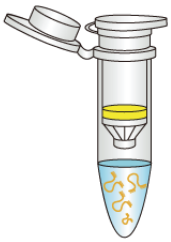
1. Cell lysis of yeast and other fungus species



2. RNA binding to membrane while contaminants remain suspended



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



4. Elution of pure total RNA which is ready for subsequent reactions

# 96 Well Blood gDNA Kit Protocol

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

**IMPORTANT BEFORE USE!**

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.

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

Additional Requirements: lyticase or zymolase, absolute ethanol, ddH2O (RNase-free and DNase-free) to prepare 70% ethanol, microcentrifuge tubes (RNase-free), pipette tips (RNase-free), ß-mercaptoethanol, 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 tube containing 600 µl of Sorbitol Buffer.

B. Yeast/Fungus in Broth
Transfer yeast/fungus cells (up to 5 x $10^7$) in broth to a 1.5 ml microcentrifuge tube. Centrifuge for 10 minutes at 5,000 x g then discard the supernatant. Re-suspend the cells in 600 µl of Sorbitol Buffer.

Add 200 U of lyticase or zymolase then mix well. Incubate at 30°C for 30 minutes. Centrifuge the mixture for 10 minutes at 2,000 x g to form a spheroplast pellet. Discard the supernatant.

**2. Cell Lysis**
Add 300 µl of RB Buffer and 3 µl ß-mercaptoethanol (or 6 µl of freshly prepared 2M Dithiothreitol in RNase Free Water) to the spheroplast pellet then vortex to mix. Incubate at room temperature for 5 minutes then centrifuge at 14-16,000 x g for 2 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube (RNase-free).

**3. RNA Binding**
Add 500 µl of 70% ethanol to the lysate and pipette immediately. Place a RB Column in a 2 ml Collection Tube. Transfer 500 µl of the mixture to the RB Column. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through. Transfer the remaining mixture to the same RB Column and centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through and 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 (20-30°C) then proceed with the RNA Wash step.

**4. RNA Wash**
Add 400 µl of W1 Buffer to the RB Column then centrifuge at 14-16,000 x g for 1 minute. 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 14-16,000 x g for 1 minute. 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. 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 14-16,000 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 37°C 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 65°C 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 RNA Pure Kit instead of stopping the reaction with EGTA.

# rYeast Total RNA Mini Kit Functional Test Data

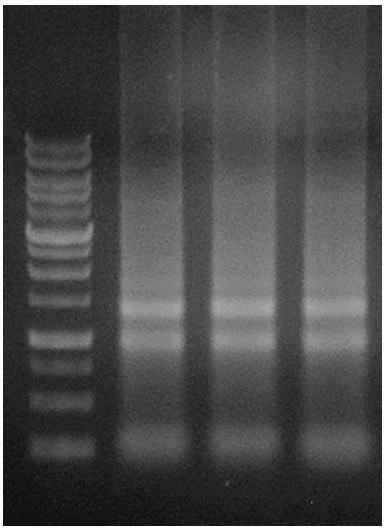



**Figure 1.**
Total RNA was extracted using the rYeast Total RNA Mini Kit. Saccharomyces cerevisiae ($5\times10^7$) was harvested by centrifugation at 5,000 x g for 10 minutes. A 5 µl aliquot of purified RNA from a 50 µl eluate was analyzed by electrophoresis on a 0.8% agarose gel.

M = 1 Kb DNA Ladder

| Test | RNA Conc. | 260/280 | 260/230 | Yield |
|---|---|---|---|---|
| 1 | 391.0 µg/ml | 2.19 | 2.48 | 19.6 µg |
| 2 | 2 389.9 µg/ml | 2.19 | 2.51 | 19.5 µg |
| 3 | 3 387.9 µg/ml | 2.20 | 2.51 | 19.4 µg |

# Troubleshooting

## Low Yield

**Clogged Column.**
Reduce the amount of starting material or separate it into multiple tubes. Centrifugation temperature must be between 20°C to 25°C. Yeast cells were not completely homogenized. Make sure lyticase or zymolase was added to Sorbitol Buffer immediately prior to use.

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

**RNA Degradation.**
The harvested sample should be stabilized immediately prior to use. Disposable plasticware & automatic pipettes should be sterile (RNase-free) & used only for RNA procedures. Non-disposable glassware or plasticware should be sterile (RNase-free).

# Related DNA/RNA Extraction Products

| Total RNA Extraction | | |
|---|---|---|
| **Product** | **Package Size** | **Catalogue Number** |
| Total RNA Mini Kit (Blood/Cell) | 50/100/300 preps | IB47321/322/323 |
| Total RNA Mini Kit (Tissue) | 50/100/300 preps | IB47301/302/303 |
| Total RNA Mini Kit (Plant) | 50/100/300 preps | IB47341/342/343 |
| rBAC Mini RNA Bacteria Kit | 100/300 preps | IB47421/422 |
| rYeast Total RNA Mini Kit | 100/300 preps | IB47411/412 |
| miRNA Isolation Kit | 100 preps | IB47371 |
| IBI Isolate | 100/200 rxns | IB47601/302 |
| **Plasmid DNA Extraction** | | |
| **Product** | **Package Size** | **Catalogue Number** |
| I-Blue Mini Plasmid Kit | 100/300 preps | IB47171/172 |
| I-Blue Mini Plasmid Endo Free Kit | 100 preps | IB47176 |
| I-Blue Midi Plasmid Kit | 25 preps | IB47181 |
| I-Blue Midi Plasmid Kit | 25 preps | IB47191 |
| Midi Fast Ion Plasmid Kit | 25 preps | IB47111 |
| Midi Fast Ion Plasmid Kit (Endotoxin Free) | 25 preps | IB47113 |
| Maxi Fast Ion Plasmid Kit | 10/25 preps | IB47121/122 |
| Maxi Fast Ion Plasmid Kit (Endotoxin Free) | 10/25 preps | IB47124/125 |
| 96 Well Plasmid Kit | 4/10 x 96 preps | IB47151/152 |
| **Post Reaction DNA Extraction** | | |
| **Product** | **Package Size** | **Catalogue Number** |
| Gel/PCR DNA Fragments Extraction Kit | 100/300 preps | IB47020/030 |
| Small DNA Fragments Extraction Kit | 100/300 preps | IB47061/062 |
| 96 Well PCR Cleanup Kit | 4/10 x 96 preps | IB47040/050 |
| **Genomic DNA Extraction** | | |
| **Product** | **Package Size** | **Catalogue Number** |
| Genomic DNA Mini Kit (Blood/Cultured Cell) | 100/300 preps | IB47201/202 |
| Genomic DNA Mini Kit (Tissue) | 50/300 preps | IB47221/222 |
| gMAX Mini Kit (Blood/Tissue) | 100/300 preps | IB47281/282 |
| Genomic DNA Mini Kit (Plant) | 100 preps | IB47230 |
| gSWAB Mini Genomic DNA Kit | 100/300 preps | IB47276/277 |
| gBAC Mini DNA Bacteria Kit | 100/300 preps | IB47291/292 |
| gYEAST Genomic DNA Kit | 100/300 preps | IB47266/267 |
| 96 Well Blood Genomic DNA Extraction Kit | 4/10 x 96 preps | IB47251/252 |
| gPURE Cell DNA Isolation Kit | 100/1000 rxns | IB47431/432 |
| **DNA/RNA/Protein Extraction** | | |
| **Product** | **Package Size** | **Catalogue Number** |
| DNA/RNA/Protein Extraction Kit | 50/100 preps | IB47701/702 |

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