Cat. # 9089

For Research Use

TaKaRa

# Yeast Processing Reagent (for total RNA preparation)

Product Manual

v202102Da

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## I. Description

Yeast Processing Reagent (for total RNA preparation) is a pretreatment reagent for use in combination with NucleoSpin RNA or RNAiso Plus to isolate high-purity total RNA from yeasts. The pretreatment of yeast cells with this product is comprised of two parts: washing and recovery of yeast cells by centrifugation; and, degradation of yeast cell wall by Yeast Processing Enzyme Solution (cell wall degradation enzyme). The pretreatment with this product requires no complicated procedures using glass beads or liquid nitrogen. Furthermore, no organic solvent is needed when used in combination with NucleoSpin RNA.

The product enables isolation of high-purity total RNA which can be used in a variety of RNA-related genetic experiments (e.g., RT-PCR), from general yeasts (e.g., *Saccharomyces, Candida*, and *Pichia*). This product can also be used to isolate total RNA from long-time cultured yeast cells or frozen yeast cells. Moreover, it offers the advantage of a shorter experimental time when used to isolate total RNA from fresh yeast cells in logarithmic growth phase.

RNA Isolation Reagents
Please use NucleoSpin RNA or RNAiso Plus in combination with this product.
Compatibilities with other RNA isolation products have not been established.

## II. Components (20 preps)

| | | |
|---|---|---|
| 1. | Yeast Processing Buffer | 1.6 ml |
| 2. | Yeast Processing Enzyme Solution*1 | 160 µl |
| 3. | RNase-free DNase I*2 | 120 µl |
| 4. | 10X DNase I Buffer*2 | 80 µl |

＊1 Yeast Processing Enzyme Solution contains an enzyme. Avoid excessive stirring or other treatment that may cause the loss of enzyme's activity.

＊2 This item is intended for use with RNAiso Plus in total RNA isolation.

## III. Materials Required but not Supplied

### 1. Reagents

- NucleoSpin RNA (Cat. #740955.10/.50/.250) or RNAiso Plus (Cat #9108/9109)
- Reagent grade ethanol (>99%)
- RNase-free water
- 1 M DTT solution or 2-mercaptoethanol (2-ME)
  - 2-ME is toxic; handle and dispose with care
  - Use with NucleoSpin RNA in total RNA isolation
- Sterile purified, ice chilled water
- chloroform, isopropanol
  - Use with RNAiso Plus in total RNA isolation

### 2. Materials

- Microcentrifuge
- Centrifuge tubes
- Temperature-controlled bath (for incubation at 30℃ and 37℃)

## IV. Storage

−20℃

## V. Protocol

1. Transfer 2 − 5 x $10^7$ cells (in the case of haploid *Saccharomyces cerevisiae*) of yeast into centrifuge tubes and centrifuge at 10,000 rpm for 2 min at 4℃.
   - Use fresh yeast cells in logarithmic growth phase.
   - This product can also be used to isolate RNA from long-time cultured yeast cells or frozen yeast cell pellets; however, the yield may be low. Moreover, the purity and quality of RNA obtained may be inferior depending on the culture condition, or the frozen storage condition or duration. Long-term storage of frozen yeast cells is not recommended.
   - In frozen yeast cell pellets, quickly perform the protocol from Step 5.
2. Carefully remove as much supernatant as possible with a micropipette.
3. Add 1 ml of sterile purified, ice chilled water to tubes, resuspend well by pipetting, and centrifuge at 10,000 rpm for 2 min at 4℃.
4. Carefully remove as much supernatant as possible with a micropipette.
5. Add 80 µl of Yeast Processing Buffer and mix gently by pipetting.
6. Add 8 µl of Yeast Processing Enzyme Solution and mix gently by pipetting.
7. Incubate the mixture at 30℃ for 30 − 60 min. During the incubation, mix every 10 − 20 min by tapping lightly.
   - When using freshly cultured yeast cells in logarithmic growth phase, it is possible to isolate RNA with a 10 min incubation at 37℃.
   - The optimum incubation time may differ depending on the yeast strain, culture condition, and number of cells. Optimization of incubation time in advance is recommended when using a different strain, culture condition, or number of cells.
   - When using a frozen yeast cell pellet, incubate at to 30℃ for 10 − 30 min.
8. Isolate total RNA from the yeast cell mixture obtained in Step 7 using NucleoSpin RNA or RNAiso Plus.
   - Modify the protocol of NucleoSpin RNA or RNAiso Plus as indicated by the underlined portions in VI. Workflow in this manual. Refer to the following sections in the instruction manual for details of the protocol.
   - NucleoSpin RNA : refer to 5 Protocols section 5.1, step 2-9 in NucleoSpin RNA manual.
     - For DNase I treatment, use rDNase RNase-free (Lyophilized) and Reaction Buffer for rDNase supplied in NucleoSpin RNA.

**Note :** For RNA purification, please use NucleoSpin RNA or RNAiso Plus. Compatibility of the Yeast Processing Reagent Kit components with other RNA isolation products has not been established.

## VI. Workflow for NucleoSpin RNA

Also refer to 5 Protocols, section 5.1, step 2-9 in NucleoSpin RNA manual. Modifications in the protocol are underlined.

Yeast cell mixture from section V-7

↓ ← Add 350 μl of RA1 (containing 1 M DTT or 2-ME)*

* Dispense the required amount of RA1 and add 20 μl of 1 M DTT or 10 μl of 2-mercaptoethanol (stock) for every 1 ml of RA1.

Vortex for 2 - 3 min. And transfer the solution in NucleoSpin Filter (violet ring) set in Collection Tube (2 ml)

↓ Centrifuge at 11,000 *g* for 1 min

Transfer the Lysate to a new centrifuge tube

↓ ← Add 350 μl of ethanol(70%)

Vortex for >15 sec, briefly spin down for a few sec

↓

lysate

↓

Pipette the lysate up and down 2 – 3 times and load the NucleoSpin RNA Column (light blue ring) placed in a Collection Tube.

Note: Maximal loading capacity of NucleoSpin RNA Column is 750 μl. Repeat the procedure if larger volumes are to be processed. (Transfer the entire lysate along with aggregates, if any, into the column)

↓ Centrifuge at 11,000 *g* for 30 sec

Change in a new Collection Tube（2 ml）

↓ ← Add 350 μl MDB (Membrane Desalting Buffer)

↓ Centrifuge at 11,000 *g* for 1 min

↓ ← Add 95 μl of DNase I solution with the composition as follows:
( Using the rDNase I in the NucleoSpin RNA)
- Reaction Buffer for rDNase (90 μl)
- Reconstituted rDNase* (10 μl)

* Prepare according to the protocol of NucleoSpin RNA

Incubate at room temperature for 15 min

↓ ← [1st wash] Buffer RAW2 200 μl

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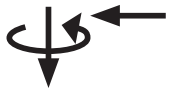
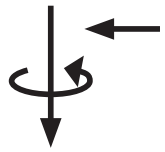
Centrifuge at 11,000 *g* for 30 sec

Change into a new Collection Tube（2 ml）



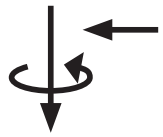
Add 600 µl of RA3 added with EtOH [2nd wash]
Centrifuge at 11,000 *g* for 30 sec

Discard flow-through



Add 250 µl of RA3 added with EtOH [3rd wash]
Centrifuge at 11,000 *g* for 2 min

Change into a nuclease-free Collection Tube (1.5 ml)



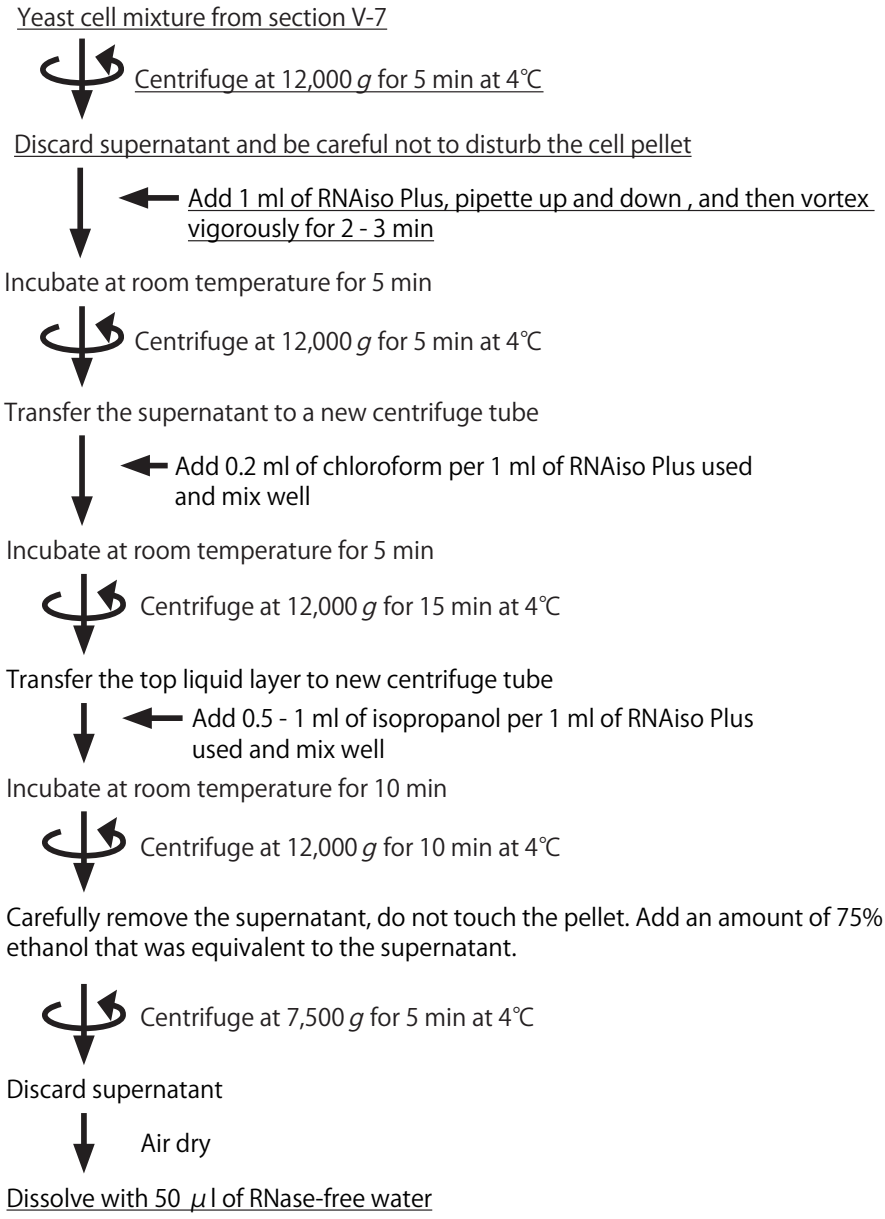
Add 60 µl of RNase-free $H_2O$
Centrifuge at 11,000 *g* for 1 min

Purified total RNA

**Workflow for RNAiso Plus**

Refer to RNAiso Plus manual. : V. Protocol and VI. RNA Extraction Flowchart. Modifications in the protocol are underlined.

**Note:** RNAiso Plus is not available in all geographic locations. Check for availability in your area.




＊ When genomic DNA is contaminated in purified RNA, DNase I treatment is recommended by using RNase-free DNase I and 10X DNase I Buffer supplied in this kit. (Refer to VIII. Appendix)

## VII. Experimental Examples

### 1. Total RNA isolation from *Saccharomyces cerevisiae* (AH109) using NucleSpin RNA.

- Total RNA was isolated from the *S. cerevisiae* (AH109) cultures (YPD medium, 1 ml) using NucleoSpin RNA.
- RNA purity and yield were determined.
- 0.5 μg of RNA was loaded onto agarose gel electrophoresis.

Table 1. Yield and Purity of Total RNA from *S cerevisiae*

| Kit Used | Yeast Cell Number | Yield (μg) | $OD_{260}/OD_{280}$ |
|---|---|---|---|
| NucleoSpin RNA | $3.6 \times 10^7$ | 32.7 | 2.2 |

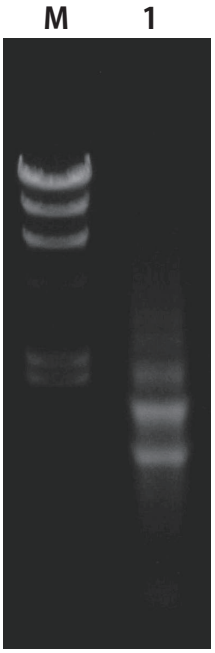



Figure 1. Electrophoresis of total RNA Isolated from *S. cerevisiae* (AH109).
M : λ-*Hin*d III digest 100 ng
Lane 1 : *S. cerevisiae* (AH109) 0.5 μg

Result : total RNA with absorbance at 260/280 nm of >2.0 was efficiently isolated from yeast strain.

**2. Total RNA isolation from *S. cerevisiae* (AH109) using RNAiso Plus***

＊ RNAiso Plus is not available in all geographic locations. Check for availability in your area.

- Total RNA was isolated according to the kit protocol from the culture (YPD medium, 1 ml) of *S. cerevisiae* (AH109).
- RNA purity and yield were determined.
- 0.5 μg of RNA was loaded onto an agarose gel electrophoresis.

Table 2. Yield and Purity of Total RNA from *S. cerevisiae*

| Kit Used | Yeast Cell Number | Yield (μg) | $OD_{260}/OD_{280}$ |
|---|---|---|---|
| RNAiso Plus | $4.8 \times 10^7$ | 57.4 | 2.2 |

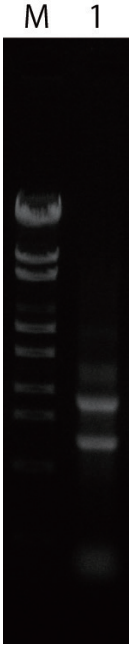



Figure 2. Electrophoresis of Total RNA Isolated from *S. cerevisiae* (AH109).
M : λ-*Eco*T14 I digest, 100 ng
Lane 1 : *S. cerevisiae*（AH109）0.5 μg

Result : Total RNA with absorbance at 260/280 nm of >2.0 were efficiently isolated using RNAiso Plus.

## VIII. Appendix

DNase I treatment for removal of contaminated DNA in total RNA purified with RNAiso Plus.

1. Prepare the following reaction mixture.

| | |
|---|---|
| Total RNA | 20 - 50 μg |
| 10X DNase I Buffer *1 | 5 μl |
| RNase-free DNase I*1 | 2 μl |
| RNase Inhibitor*2 | 20 U |
| RNase-free water | up to 50 μl |

   * 1 The reagent attached in this product can be used. DNase I (RNase-free) (Code #2270A) can also be used.
   * 2 Recombinant RNase Inhibitor (Code #2313A) is required.

2. Incubate for 20 – 30 min at 37℃.
3. Perform one of the following procedures to inactivate DNase I.
   A. Heat treatment
      (1) Add 2.5 μl of 0.5 M EDTA, incubate at 80℃ for 2 min.
      (2) Fill up reaction volume to 100 μl with RNase-free water.
   B. Phenol/Chloroform extraction
      (1) Add 50 μl of RNase-free water and 100 μl of phenol/chloroform/isoamyl alcohol (25 : 24 : 1) , and vortex.
      (2) Centrifuge at 12,000 rpm for 5 min at room temperature, then transfer the upper layer to a new tube.
      (3) Add equal amount of chloroform/isoamyl alcohol (24：1) and vortex.
      (4) Centrifuge at 12,000 rpm for 5 min at room temperature, then transfer upper layer to new tube.
4. Add 10 μl of 3M sodium acetate and 250 μl of ethanol, and then mix. Keep it for 20 min at -80 ℃.
5. Centrifuge at 12,000 rpm for 10 min at 4℃. Remove the supernatant.
6. Wash the precipitate with cold 70% ethanol. Centrifuge at 12,000 rpm for 5 min at 4℃ and remove the supernatant.
7. Dry the precipitate.
8. Dissolve the precipitate in a appropriate amount of RNase-free water. Confirm the genomic DNA removal by electrophoresis and measure RNA concentration. When the genomic DNA is not removed completely, increase the amount of DNase or extend reaction time.

## IX. Troubleshooting

1. Low quantity of total RNA
   - A large number of yeast cells.
     When using more than $2 - 5 \times 10^7$ cells (in case of *Saccharomyces cerevisiae*), degradation of cell wall may be incomplete with the enzyme contained in the Yeast Processing Enzyme Solution, resulting in a low yield of total RNA. Use no more than the number of yeast cells that can be treated. The number of yeast cells that can be treated may differ depending on the yeast species (genus). When isolating total RNA from a yeast of another species, the treatable number of yeast cells needs to be optimized in advance.
   - A small number of yeast cells.
     When isolating total RNA from yeast cells in a number significantly less than $2 - 5 \times 10^7$ (in case of *Saccharomyces cerevisiae*), yield and purity of the total RNA preparation may decrease. To isolate total RNA at a stable recovery rate, use yeast cells in a number close to the prescribed count. The number of yeast cells that can be treated may differ depending on a yeast species (genus). When isolating total RNA from a yeast of another genus, the treatable number of yeast cells needs to be optimized in advance.
   - Total RNA was isolated from a yeast incompatible with this product.
     Yeast cell wall degradation is achieved by enzyme in the Yeast Processing Enzyme Solution in this kit. If this enzyme is unable to degrade the cell wall of a yeast, the quantity of total RNA may be significantly lower.
2. Degradation of total RNA
   - The optimum pretreatment time may vary depending on the yeast species, culture condition, or the state of the sample (fresh yeast cells in logarithmic growth phase, yeast cells cultured for a long period, or yeast pellet stored frozen, etc.). If the yeast species, culture condition, or the state of the sample is different, the treatment time needs to be optimized in advance.
   - The quality and purity of total RNA isolated from frozen yeast pellets are highly susceptible to the effects of the frozen condition and storage duration. In such cases, prepare total RNA from fresh yeast cells in logarithmic growth phase. Long-term storage of cells at frozen state is not recommended.
3. Total RNA is contaminated with genomic DNA
   - When using NucleoSpin RNA, make sure to perform a DNase treatment on a column using the Reconstituted rDNase* and Reaction Buffer for rDNase supplied in NucleoSpin RNA (*Prepare according to the protocol of NucleoSpin RNA even after DNase I treatment on a column, a high level of genomic DNA contamination may still persist depending on the yeast species (genus), culture condition, or excess cell number of yeast.
4. In addition, refer to the section on troubleshooting provided in the instruction manual for NucleoSpin RNA or RNAiso Plus.

## X. Related Products

NucleoSpin RNA (Cat. #740955.10/.50/.250)
RNAiso Plus(Cat #9108/9109)

Dr. GenTLE™ (from Yeast) High Recovery (Cat. #9082)

Recombinant DNase I (RNase-free) (Cat. #2270A/B)

Recombinant RNase Inhibitor (Cat. #2313A/B)