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I. Description

Yeast Processing Reagent (for total RNA preparation) is a pretreatment reagent for use in combination with NucleoSpin® RNA II 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 process 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 II.

The pretreatment with this 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 II or RNAiso Plus* in combination with this product. Compatibilities with other RNA isolation products have not been established.

* : Not available in all geographic locations. Check for availability in your region.

II. Kit Components (20 preps)

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

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

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

III. Materials Required but not Supplied

1. Reagents
   - NucleoSpin® RNA II (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 II in total RNA isolation
   - Sterilized, ice chilled water
   - chloroform, isopropanol
     - Use with RNAiso Plus in total RNA isolation

*: Not available in all geographic locations. Check for availability in your region.

2. Materials
   - Microcentrifuge
   - Centrifuge tubes
   - Temperature-controlled bath (for incubation at 30°C and 37°C)
V. Protocol

1. Transfer yeast culture containing $2 - 5 \times 10^7$ cells (in the case of haploid *Saccharomyces cerevisiae*) into centrifuge tubes and centrifuge at 10,000 rpm for 2 minutes at 4°C.
   - 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 samples may be inferior depending on the culture condition, or the frozen storage condition or duration. Long-term storage in a frozen state is not recommended.
   - Using frozen yeast cell pellets, place the frozen pellets on ice and quickly perform the procedures from Step 5.

2. Carefully remove as much supernatant as possible with a micropipette.

3. Add 1 ml of sterilized, ice chilled water to the yeast cell pellet, resuspend well by pipetting, and centrifuge at 10,000 rpm for 2 minutes at 4°C.

4. Carefully remove as much supernatant as possible with a micropipette.

5. Add 8 μl of Yeast Processing Enzyme Solution and mix gently by pipetting.

6. Add 8 μl of Yeast Processing Buffer and mix gently by pipetting.

7. Incubate the mixture at 30°C for 30 – 60 minutes. During the incubation, mix every 10–20 minutes by tapping lightly.
   - When using freshly cultured yeast cells in logarithmic growth phase, it is possible to isolate RNA with a 10-minute incubation at 37°C. (Refer to VII. Experimental Example 5)
   - 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, set the incubation condition to 30°C for 10–30 minutes. (Refer to VII. Experimental Example 4)

8. Isolate total RNA from the yeast cell mixture obtained in Step 7 using NucleoSpin® RNA II or RNAiso Plus.
   - Modify the protocol of NucleoSpin® RNA II 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 II: Also refer to 5 protocols section5-4, Step2-4 and section 5-1, step5-9 in Nucleospin® RNA II manual.
     - For DNase I treatment, use rDNase RNase-free (Lyophilized) and Reaction Buffer for rDNase supplied in this kit.

Note: For RNA purification, please use NucleoSpin® RNA II 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 II

Also refer to 5 protocols section 5-4, Step 2-4 and section 5-1, step 5-9 in Nucleospin RNA II manual. Modifications in the protocol are underlined.

Pretreated yeast cell mixture

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

*: Dispense the required amount of RA1 and add 40 μl of 1 M DTT or 10 μl of 2-mercaptoethanol (stock) for every 1 ml of RA1.

Vortex for 2 - 3 minutes. Reduce viscosity and turbidity of the solution by filtration through NucleoSpin® Filters (violet rings). Place NucleoSpin® Filters in Collection Tubes (2 ml)

Centrifuge at 11,000 Xg 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.

Prepared lysate

For each preparation take one NucleoSpin® RNA II Column (light blue ring) placed in a Collection Tube. Pipette lysate up and down 2–3 times and load the lysate to the column.

Place the column in a new Collection Tube (2 mL).

Maximal loading capacity of NucleoSpin® RNA II Columns 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 Xg for 30 sec

Change the Collection Tube (2 ml)

Add 350 μl MDB (Membrane Desalting Buffer)

Centrifuge at 11,000 Xg for 1 min.

Add 95 μl of DNase I solution with the composition as follows:

((DNase I treatment should be performed using the rDNase I in the Nucleospin® RNA II))

- Reaction Buffer for rDNase (90 μl)
- Reconstituted rDNase* (10 μl)

*: Prepare according to the protocol of NucleoSpin® RNA II

Incubate at room temperature for 15 min

[1st wash] Buffer RA2 200 μl

(Continued on next page)
(Continued from previous page)

Centrifuge at 11,000X g for 30 sec.

Change the Collection Tube (2 ml)

Add 600 μl of RA3 added with EtOH [2nd wash]

Centrifuge at 11,000X g for 30 sec.

Discard flow-through

Add 250 μl of RA3 added with EtOH [3rd wash]

Centrifuge at 11,000X g for 2 min.

Change the nuclease-free collection tube (1.5 ml)

Add 60 μl of RNase-free dH2O

Centrifuge at 11,000X g for 1 min.

Purified Total RNA
Workflow for RNAiso Plus
Also refer to RNAiso Plus manual. :RNA extraction flowchart and VII. Analysis of RNA purity.

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

Pretreated yeast cell mixture

Centrifuge at 12,000X g for 5 min at 4°C.

Discard supernatant and be careful not to disturb the cell pellet.

Adding 50 μl of RNAiso Plus, Pipette up and down until pellet, homogenize by vortex vigorously for 2 - 3 min.

incubate at room temperature for 5 min

Centrifuge at 12,000X g for 5 min at 4°C.

Transfer the supernatant to a new centrifuge tube

Add 0.2 ml of chloroform per 1 ml of RNAiso Plus used for homogenization and mix well.

incubate at room temperature for 5 min

Centrifuge at 12,000X g for 15 min at 4°C.

Transfer the top liquid layer to new centrifuge tube

Add 0.5 - 1 ml of isopropanol per 1 ml of RNAiso Plus used for homogenization and mix well.

incubate at room temperature for 10 min

Centrifuge at 12,000X g for 10 min at 4°C.

Carefully remove the supernatant, do not touch the pellet. Add an amount of 75% cold ethanol that was equivalent to the supernatant.

Centrifuge at 7,500X g for 5 min at 4°C.

Discard supernatant and keep precipitate.

Air dry

Dissolve with 50 μl of RNase-free water.

* For DNase I treatment, use RNase-free DNase I and 10× DNase I Buffer supplied in this kit. (Refer to VII. Appendix)
VII. Experimental Examples

1. Total RNA isolation from *Saccharomyces cerevisiae* (AH109) using NucleSpin® RNA II.
   - Total RNA was isolated from the cultures of *S. cerevisiae* (AH109) cultures (YPD medium, 1 ml) using the Nucleospin RNA II.
   - RNA purity and yield were determined.
   - 0.5 μg of RNA sample was loaded onto an agarose gel for electrophoresis.

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

<table>
<thead>
<tr>
<th>Kit Used</th>
<th>Yeast Cell Number</th>
<th>Yield (μg)</th>
<th>OD₂₆₀/OD₂₈₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleospin® RNA Kit</td>
<td>3.6 x 10⁷</td>
<td>32.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Figure 1. Electrophoresis of total RNA Isolated from *S. cerevisiae* (AH109).

M : λ -Hind III I digest 100 ng
Lane 1 : *S. cerevisiae* (AH109) 0.5 μg

Result: A total RNA preparation with absorbance at 260/280 nm of >2.0 was efficiently isolated from this 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 region.

- 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 sample was loaded onto an agarose gel for electrophoresis.

<table>
<thead>
<tr>
<th>Kit Used</th>
<th>Yeast Cell Number</th>
<th>Yield (μg)</th>
<th>OD(<em>{260}/OD</em>{280})</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNAiso Plus</td>
<td>(4.8 \times 10^7)</td>
<td>57.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Figure 2. Electrophoresis of Total RNA Isolated from *S. cerevisiae* (AH109).

M: \(\lambda\) -EcoT14 I digest, 100 ng
Lane 1: RNAiso Plus

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

Contaminating genomic DNA is removed by a rDNase solution

Following procedures to DNase I

1. Prepare the following reaction mixture.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RNA</td>
<td>20 - 50 μg</td>
</tr>
<tr>
<td>10X DNase I Buffer (supplied)</td>
<td>5 μl</td>
</tr>
<tr>
<td>Recombinant DNase I (RNase-free)</td>
<td>2 μl (10 units)</td>
</tr>
<tr>
<td>Ribonuclease Inhibitor</td>
<td>20 U</td>
</tr>
<tr>
<td>RNase-free water</td>
<td>up to 50 μl</td>
</tr>
</tbody>
</table>

*1: The reagent attached to this product can use. DNase I (RNase-free) (Code #2270A) can also be used.
*2: Separately, 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) Increase reaction volume to 100 μl with DEPC treated water.

B. Phenol/Chloroform extraction
   (1) Mix 50 μl of DEPC treated water and 100 μl of phenol/chloroform/isoamyl alcohol (25 : 24 : 1) together.
   (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 mix.
   (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 chilled 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 chilled 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 suitable amount of RNase-free water. Confirm the genomic DNA is removed completely by electrophoresis and measure the RNA concentration. When the genomic DNA is not removed completely, increase the amount of enzyme or extend reaction time.
IX. Troubleshooting

1. Low quantity of total RNA isolated

   • A large number of yeast cells was used. When using more than $2 \times 5 \times 10^7$ cells (in case of \textit{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 (genus). When isolating total RNA from a yeast of another genus, the treatable number of yeast cells needs to be optimized in advance.

   • A small number of yeast cells was used. When isolating total RNA from yeast cells in a number significantly below the count of $2 \div 5 \times 10^7$ (in case of \textit{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 (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 an enzyme in the Yeast Processing Enzyme Solution supplied in this kit. If this enzyme is unable to completely degrade the cell wall of a yeast, the quantity of total RNA obtained may be significantly lower.

2. Tendency of the isolated total RNA to degrade

   • The optimum pretreatment time may vary depending on the yeast (genus), culture condition, or the state of the sample used (fresh yeast cells in logarithmic growth phase, yeast cells cultured for a long period, or yeast pellet stored frozen, etc.). If the yeast (genus), culture condition, or the state of the sample used is different, the treatment time needs to be optimized in advance.

   • The quality and purity of total RNA preparations isolated from frozen yeast pellets, in particular, are highly susceptible to the effects of the frozen condition and storage duration. In such cases, prepare fresh yeast cells in logarithmic growth phase. Long-term storage at frozen state is not recommended.

3. Total RNA contaminated with genomic DNA

   • When using Nucleospin® RNA II to isolate total RNA, make sure to perform a DNase treatment on a column using the Reconstituted rDNase® and Reaction Buffer for rDNase supplied in Nucleospin® RNA II (Prepare according to the protocol of NucleoSpin® RNA II). Nevertheless, even after a DNase I treatment on a column, a high level of genomic DNA contamination may still persist depending on the yeast (genus), culture condition, or whether the number of yeast cells used exceeded the treatment capacity.

4. In addition, refer to the section on troubleshooting provided in the instruction manual for Nucleospin® RNA II or RNAiso plus.
X. Related Products

- Nucleospin® RNA II (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)

*: Not available in all geographic locations. Check for availability in your region.
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