Ver. 23071

ODP418

Fungal RNA Extraction Kit

For extraction of total RNA from fungal samples

Origin

ISO 13485:2016 ISO 9001:2015

Fungal RNA Extraction Kit

(Spin Column)

Kit Contents

	50 Preps
Buffer RS	50mL
Buffer LY	40mL
Lyticase (10U/μL)	500μL
Buffer RD	13mL
Buffer RW	15mL
RNase-free ddH_2O	10mL
RNase-free spin column CR3 & Collection tube (2mL)	50
Hand book	1

Storage

Buffer RS

For short term (≤1 month): At room temperature, protected from light

For long term (≥1 month): At 2-8°C, protected from light

Store Buffer LY at 2–8°C and Lyticase (10 U/μL) at –20°C.

Other reagents: At room temperature

Note: Buffer RS may be transported at room temperature

Introduction

Fungal RNA Extraction kit uses a new technology based on guanidine thiocyanate/phenol method. It contains a unique Buffer RS that minimizes the contamination of genomic DNA and protein. Fungal RNA Extraction kit can efficiently extract high pure RNA from blood, cells, tissues and plant samples in one hour. The extracted RNA is ready-to-use in downstream applications such as: PCR, gene chips assay, northern blot, dot blot, poly A screening, *in vitro* transcript and molecular cloning.

Notes for avoiding RNase contamination

- Change gloves regularly.
- Use RNase-free plasticwares or glasswares to avoid cross contamination
- To remove RNase, the glassware can be roasted at 150°C for 4 hours, while plastic can be dipped in 0.5M NaOH for 10 minutes, washed by RNase-free ddH_2O thoroughly and sterilized.
- Use RNase-free ddH_2O. (Add DEPC into water in clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 minutes to remove any trace of DEPC).

Materials Required but Not Provided

- β-Mercaptoethanol
- Chloroform
- Ethanol (99-100%)

Protocol

Buffer RD and Buffer RW are supplied as a concentrate. Before using for the first time, add ethanol (96-100%) as indicated on the bottle to obtain a working solution.

1. Harvest 1-5×10^9 cells by centrifugation and remove culture medium. Add 600µL Buffer LY, 100µL β-Mercaptoethanol and 10µL Lyticase to the pellet. Incubate the sample at room temperature for 30 minutes. Occasionally mix the content by inverting the tube.
   Note: The volume of Buffer LY, Lyticase and β-Mercaptoethanol will vary with cell number and type of cells
2. Centrifuge the sample at 4,000 rpm for 5 minutes and remove the supernatant.
3. Add 1mL of Buffer RS to the pellet. Do not wash cells before addition of Buffer RS to avoid increased chance of mRNA degradation. Samples from some yeast may need to be homogenized by using a power homogenizer.
4. Incubate sample at room temperature for 10 minutes, to permit complete dissociation of the nucleoprotein complex.
   Optional step:

- **For difficult to lyse sample, incubate at 65°C for 10 minutes.**
- **Centrifuge the sample at 12,000 rpm for 10 minutes at 4°C. Transfer the supernatant to a fresh microcentrifuge tube.**
  Note: When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional centrifugation may be required to remove insoluble material from the samples. RNA remains in the upper aqueous phase after centrifugation. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clear aqueous phase part for next step.

5. Add 200µL of chloroform per 1mL Buffer RS used for homogenization. Cap the tube securely mix the contents by inverting the tube for 15 seconds. Incubate for 3 minutes at room temperature.
6. Centrifuge the sample for 10 minutes at 12,000 rpm at 4°C. The mixture separates into a lower phenol chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Pipette the aqueous phase out into a new tube.
7. Add the 0.5 volume ethanol (96%-100%) to the aqueous phase. Mix thoroughly by inversion (precipitate may appear in this step).
   Note: Add 0.5mL ethanol if the initial volume of RS is 1mL.

8. Transfer the sample, including any precipitate that may have formed, to an RNase- free spin column CR3 placed in a 2mL RNase-free collection tube. Close the lid gently, and centrifuge at 12,000 rpm for 30 seconds at 4°C. Discard the flow-through.
   Note: Since the capacity of CR3 is 700µL, the loading-centrifugation step must be repeated for processing all the mixture from step 7.
9. Add 500µL Buffer RD to the RNase-free spin column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000rpm for 30 seconds at 4°C. Discard the flow- through.
10. Add 700µL Buffer RW to the RNase-free spin column CR3 (Ensure ethanol has been added). Close the lid gently, and centrifuge at 12,000rpm for 30 seconds at 4°C. Discard the flow- through.
11. Set the RNase-free spin column CR3 back to the collection tube. Centrifuge at 12,000rpm for 2 minutes at 4°C to dry the spin column membrane.
    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
12. Place the RNase-free spin column, CR3 in a new 1.5mL RNase- free collection tube (not supplied). Add 30-100µL RNase-free ddH_2O directly to the spin column membrane. Close the lid gently, incubate at room temperature (15–25°C) for 2 minutes. Centrifuge at 12,000rpm for 2 minutes at 4°C to elute the RNA.
    Note: The volume of elution buffer should not be less than 30µL, or it may affect recovery efficiency. To obtain higher productivity, add the solution gained from step 11 to the center of membrane again, let the columns stand for 1 minute, and then centrifuge. Extracted RNA should be stored at –80°C.

Yield

Number of cells	RNA Concentration
1-5×10^9	10-20ng/µL