CYRN10-CHANGYU EASYspin Yeast RNA Kit

Catalog number	Package
CYRN1001	50 preps

Storage and shipping: 15-25 °C; store the low-temperature component Lytic Enzyme at -20 °C. All components can be shipped at room temperature. Stable for 12 months when stored as indicated. Lytic Enzyme is a snailase glycerol stock and is viscous; handle carefully. Keep all solutions tightly capped after use.

Description

Yeast cell walls are removed with Lytic Enzyme and cells are lysed in a dedicated buffer without phenol or chloroform. The kit is suitable for efficient isolation of highly pure, high-quality total RNA from a range of yeasts. The resulting total RNA has high purity and low gDNA contamination, is free of protein and other impurities, and is suitable for RT-PCR, RT-qPCR, RNA-seq library preparation, array analysis and other downstream applications.

Kit components

Component	Storage	50 preps (CYRN1001)
Buffer SE	15-25 °C	15 ml
Lysis Buffer RLT	15-25 °C	20 ml
Deproteinization Buffer RW1	15-25 °C	35 ml
Wash Buffer RW	15-25 °C	10 ml (add ethanol before first use)
RNase-free H2O	15-25 °C	5 ml
Lytic Enzyme	-20 °C	2.5 ml
RNA spin columns and collection tubes	15-25 °C	50 sets

Lytic Enzyme is a snailase preparation from snail crop and digestive tract containing more than 20 enzymes, including cellulase, pectinase, amylase and protease, and is suitable for digesting yeast cell walls.

Features

The silica membrane is specially selected; variation in binding capacity between columns is minimal and reproducibility is high.

Fast and simple: a single sample is usually processed within 20 minutes.

Excellent RNA integrity: the typical 28S:18S ratio on agarose gels is greater than 2:1.

Repeated column washes ensure high purity; typical A260/A280 is 2.1-2.2.

The final empty centrifugation removes residual ethanol completely, so RNA can be eluted directly without opening the tube to air-dry ethanol.

Notes before starting

Note: All centrifugation steps are carried out at room temperature at 13,000 rpm.

Note: Ethanol, β-mercaptoethanol and a water bath must be supplied by the user.

Note: Lysis Buffer RLT and Deproteinization Buffer RW1 contain irritants. Wear gloves and avoid contact with skin, eyes and clothing. Rinse affected areas with plenty of water or saline.

Note: The kit removes most DNA contamination and the purified RNA generally requires no DNase I treatment. If the downstream application is very sensitive to trace DNA, treat the RNA with DNase I (CYRN45) or perform on-column DNase digestion during extraction (CYRN34).

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer RW as indicated on the label. Before use, add β-mercaptoethanol to Lysis Buffer RLT to a final concentration of 1% (for example 10 μl per 1 ml RLT); prepare fresh, and the prepared RLT can be stored at 4 °C for one month. Add β-mercaptoethanol to the required volume of Buffer SE to 0.2%.

1. Small-scale yeast culture

a. Collect 1 ml (about 10^7 cells) of logarithmic-phase yeast culture into a 1.5 ml centrifuge tube, centrifuge at 13,000 rpm for 30 seconds and remove the supernatant as completely as possible.

b. Add 100 μl of Buffer SE (confirmed to contain 0.2% β-mercaptoethanol) and resuspend the cells gently by pipetting. Add about 20 μl of Lytic Enzyme stock, mix thoroughly by inversion and incubate at 37 °C for 15-30 minutes to digest the cell wall, inverting several times.

If cell wall digestion is incomplete and yield is low, increase the amount of Lytic Enzyme, extend the digestion time or raise the temperature to 45 °C. Common yeasts such as Pichia pastoris and Saccharomyces cerevisiae work well; for yeasts poorly digested by Lytic Enzyme, use 0.5 mm glass beads with vortexing, repeated freeze-thaw or lyticase.

c. Add 380 μl of Lysis Buffer RLT (confirmed to contain 1% β-mercaptoethanol), mix by pipetting and vortex vigorously for 20 seconds. If obvious clumps or insoluble material remain, centrifuge at 13,000 rpm for 3 minutes and transfer all of the supernatant to a new tube.

d. Add 280 μl of absolute ethanol, mix immediately by pipetting and do not centrifuge.

e. Proceed to step 3.

2. Medium-scale yeast culture

a. Collect 2-3 ml (about 3×10^7 cells) of logarithmic-phase yeast culture into a 1.5 ml centrifuge tube (if more than 1.5 ml, collect in two rounds in the same tube), centrifuge at 13,000 rpm for 30 seconds and remove the supernatant as completely as possible.

b. Add 300 μl of Buffer SE (confirmed to contain 0.2% β-mercaptoethanol) and resuspend the cells gently. Add 50 μl of Lytic Enzyme stock, mix by inversion and incubate at 37 °C for 15-30 minutes, inverting several times.

c. Add 350 μl of Lysis Buffer RLT (confirmed to contain 1% β-mercaptoethanol), mix by pipetting and vortex vigorously for 20 seconds.

d. Add an equal volume of 70% ethanol prepared with DEPC-treated water (about 350 μl) and mix immediately by pipetting; do not centrifuge. If 70% ethanol is unavailable, 0.5 volume of absolute ethanol (about 200 μl) can be used.

e. Proceed to step 3.

3. Load the mixture onto a spin column placed in a collection tube and centrifuge at 13,000 rpm for 30-60 seconds. Discard the flow-through.

4. Add 700 μl of Deproteinization Buffer RW1, leave at room temperature for 1 minute, centrifuge at 13,000 rpm for 30 seconds and discard the flow-through.

5. Add 500 μl of Wash Buffer RW (check that ethanol has been added), centrifuge at 13,000 rpm for 15 seconds and discard the flow-through.

6. Repeat step 5 once.

7. Return the column to the collection tube and centrifuge at 13,000 rpm for 2 minutes. This empty centrifugation removes residual wash buffer, since residual ethanol can inhibit downstream reactions.

8. Transfer the column to a new 1.5 ml centrifuge tube. Apply 30-50 μl of RNase-free ddH2O to the centre of the membrane, leave for 1 minute and centrifuge at 13,000 rpm for 1 minute.

Use an elution volume of at least 30 µl; smaller volumes reduce recovery. To increase RNA concentration, prewarm the RNase-free ddH2O to 90 °C or reload the first eluate onto the column for a second elution, which increases concentration by about 10%.

9. The extracted RNA can be used directly in downstream applications or stored at -85 to -65 °C.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.