GRS total RNA Kit – Yeast & Fungus

#GK17.0100 (100 preps) | GK17s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 5x10^7 cells of a large variety of yeast and fungus species
Expected Yield : up to 30µg total RNA
(typically 20-25µg from 5x10^7 *S.cerevisiae*)
Format : spin column (certified DNase/RNase-free)
Operation Time : 20-30 minutes (from spheroplasts), within 1.5 hours from cells
Elution Volume : 50-100µl

Product: The GRS Total RNA Kit – Yeast & Fungus - provides an efficient and fast method for the purification of high-quality total RNA (including mRNA, tRNA and rRNA) from yeast and a wide variety of fungus species. Eluted purified RNA is suitable for RT-PCR, Northern Blotting, mRNA selection, cDNA synthesis, and primer extension.

The GRS Total RNA Kit – Yeast & Fungus – includes Sorbitol Buffer to reduce sample preparation and thus overall minimizes hands on time. Detergents and chaotropic salts lyse spheroplasts and denature proteins/inactivate RNase. Optional DNase treatment can be included in the protocol to remove undesired trace amounts of DNA. The buffer system is optimized to allow selective binding of RNA to the glass fiber matrix of the spin column[1]. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified RNA is subsequently eluted with RNase-free Water. The entire procedure can be completed within 20 minutes (from spheroplasts) with typical RNA yields of 20µg (from 5x10^{7}cells).

QC: The quality of the GRS Total RNA Kit – Yeast & Fungus - is tested on a lot-to-lot basis by isolating total RNA from a 5x10^7 *Saccharmoyces cerevisiae* cells, harvested from a fresh culture by centrifugation at 5,000g for 10min. Quantity and Quality are ascertained by spectroscopy and gel electrophoresis.

Caution: YF Lysis Buffer contains chaotropic salt which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

In order to prevent RNase contamination, one should use disposable plastic ware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-free and used only for RNA procedures. During handling, gloves should be worn at all times.

References:

1. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

Kit Contents	(100 preps)	(4 preps)
Sorbitol Buffer	90 ml	4.5 ml
YF Lysis Buffer	60 ml	2 ml
Wash Buffer 1	50 ml	2 ml
Wash Buffer 2*	25ml+12.5ml	1 ml
RNase-free Water	15 ml	1 ml
RNA mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	300	-
2-ml collection tube	200	8
DNase I solution	0.55 ml	-
DNase I reaction buffer	5 ml	-

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips (RNase-free)
Vortex
Water bath or Thermoblock
Ice
β-mercaptoethanol
Zymolyase

* Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottles, prior to initial use. After Ethanol has been added, mark the bottles to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.

Storage: Transport of DNase I solution and DNase I reaction buffer is carried out either with or without cooling. Upon arrival, these should be stored at -20°C. In case of DNase I solution, one should consider to prepare small aliquots, as it is not recommended to repeat thawing and freezing cycles more than 3 times. All other components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37°C followed by cooling to 25°C. Store for up to 2 years.

DNase I treatment of RNA

DNA contamination in the final RNA solution interferes with several downstream applications, such as gene expression analysis. The amount of DNA contamination in the RNA eluate can be significantly reduced by DNase I treatment of the sample. This can be conveniently done "in column" (see step 6 on page 3). We highly recommend to use the reaction buffer included in this kit, as standard DNase buffers often are incompatible with in column DNase I treatment and might compromise RNA yield and integrity. For some very sensitive applications it might be necessary to eliminate even the smallest amounts of residual DNA. In order to effectively remove any trace amounts of DNA, one should consider to treat the eluted RNA with DNase I as described hereunder.

DNA Digestion in Solution (Optional)

Mix as follows in a RNase-free microtube:

- Purified RNA (in RNase-free water): 5-40 µl
- DNase I Reaction Buffer (1x): 5 µl
- DNase I Solution : 0.5 µl for each µg of purified RNA
- RNase-free water: make up to final volume of 50 µl

Incubate at 37°C for 15-30 minutes and stop the reaction by adding 1µl of 20mM EGTA (pH 8.0) and heating at 65°C for 10 minutes.

If desired, DNase I can be removed from the reaction mixture by standard phenol extraction.

Alternatively, remove DNase I by adding 250 µl of YF Lysis Buffer and 300µl of 70% ethanol (prepared with RNase-free water) and mix well by vortexing. Transfer all of the mixture to a new RNA mini spin column and centrifuge at 14,000-16,000g for 1 minute. Discard the flow-through and proceed with step 7 on page 3. Note that following this option the total amount of RNA purifications that can be done with this kit will be reduced.

PROTOCOL FOR TOTAL RNA PURIFICATION FROM YEAST & FUNGUS

1) (preparation of spheroplasts) Transfer up to $5x10^7$ yeast or fungus cells to a 1.5-ml RNase-free microcentrifuge tube, and centrifuge for 10 minutes at 5,000g. Discard the supernatant completely and resuspend the pellet in 600µl of Sorbitol Buffer. Add 200U of Zymolyase (not included) or Lyticase (not included) and incubate at 30ºC for 30 minutes. Harvest spheroplasts by centrifugation at 3.000g for 10 min and discarding the supernatant.

2) (lysis) Add 300 µl of YF Lysis Buffer and 3µl of ß-mercaptoethanol (not included) to the spheroplasts. Incubate at room temperature for 5 minutes. Centrifuge for 2 minutes at 14,000-16,000g and transfer the supernatant to a new 1.5-ml RNase-free microcentrifuge tube.

3) Add 500µl of 70% ethanol (prepared with RNase-free ddH_2O) and mix well by shaking vigorously. In case precipitate appears, break it up by pipetting.

4) Place the RNA mini spin column in a 2-ml collection tube and transfer 500µl of the sample mixture (including any precipitate) to the column

5) Centrifuge at 14,000g-16,000g for 2 minutes. If the lysate did not pass completely through the column, increase centrifuge time until the mixture passes completely. Discard the flow-through and transfer the remaining sample to the same spin column. Centrifuge at 14,000g-16,000g for 2 minutes (or longer). Discard the collection tube and place the RNA mini spin column in a new collection tube.

6) ***[optional (see page 2)]*** Add 400 µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the RNA mini spin column back in the collection tube. Mix for each prep 45 µl of DNase I reaction buffer with 5 µl of DNase I solution in a RNase-free tube, and then pipet 50 µl to the center of each spin column. Incubate at room temperature for 10-15 minutes. **Ensure ethanol was added to Wash Buffer 2 prior to use this kit the 1st time.*

7) Add 400 µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the RNA mini spin column back in the collection tube. Add 600 µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds. **Ensure ethanol was added to Wash Buffer 2 prior to use this kit the 1st time.*

8) Discard the flow-through and add 600 µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds. **Ensure ethanol was added to Wash Buffer 2 prior to use this kit the 1st time.*

9) Discard the flow-through and place the RNA mini spin column back in the collection tube and centrifuge at 14,000g-16,000g for another 3 minutes to dry the matrix of the column.

10) Transfer the spin column to a new 1.5-ml microcentrifuge tube (RNase-free) and pipet 50 µl of RNase-free Water directly to the center of the spin column without touching the membrane. Incubate at room temperature for 1-2 minutes. Total yield can be increased by eluting with larger volume (e.g.,100 µl) whereas concentration can be increased with eluting with smaller volume (e.g., 25 µl) of RNase-free water.

11) Centrifuge for 1 minute at 14,000g-16,000g to elute purified total RNA. Discard the spin column and use RNA immediately or store at -20ºC for short term storage or at -70ºC for long term storage.

TROUBLESHOOTING

1. **Low Yield**
    - *Clogged Column*
        - **i.** Reduce the amount of sample material.
        - **ii.** Make sure that Zymolyase (or Lyticase) was added to YF Lysis Buffer immediately prior to use
    - *Incorrect RNA Elution Step*
        - **i.** Ensure that the RNase-free water is completely adsorbed after being added to the center of the spin column.

2. **Low Quality**
    - *Low performance in downstream applications*
        - **i.** Residual ethanol contamination interferes with downstream applications. Following the wash step, dry the spin column with additional centrifugation for 5 minutes or incubation at 60°C for 5 minutes in order to evaporate ethanol.
        - **ii.** RNA degradation by RNases (which can be detected by gel analysis), may be the result of improper handling of starting material.