GRS Pure RNA Kit

#GK15.0100 (100 preps) | GK15s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	up to 100µl of RNA products
Expected Yield :	up to 90% (up to 50µg)
Format :	spin column
Operation Time :	10-20 minutes
Elution Volume :	25-100µl

Product: The GRS Pure RNA Kit provides an efficient and fast method for the purification and or concentration of high-quality RNA from samples containing partial purified RNA obtained via other RNA isolation methods. The purified RNA is suitable for use in Northern Blotting and RT-PCR.

The GRS Pure RNA Kit uses a unique RNA Binding Buffer that is optimized to allow easy binding of RNA to the glass fiber matrix of the spin column[1]. Contaminants such as proteins, DNA, phenol, divalent cations, unincorporated nucleotides, and enzyme inhibitors are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified RNA is subsequently eluted by RNase-free water or TE (RNase-free). The entire procedure can be completed within 20 minutes without phenol/chloroform extraction and/or alcohol precipitation, with a typical RNA recovery of 80% to 90%.

QC: The quality of the GRS Pure RNA Kit is tested on a lot-to-lot basis by purifying RNA of various sizes from aqueous solutions, followed by subsequent agarose electrophoresis

Caution: The RNA Binding Buffer contains guanidine thiocyanate 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)
RNA Binding Buffer	60 ml	3 ml
Wash Buffer*	25ml	1 ml
RNase-free Water	6 ml	1 ml
RNA mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	200	-
2-ml collection tube	100	4

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

* Add Ethanol (96%-100%) [not included] to the Wash Buffer, as indicated on the bottle, 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: 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.

PROTOCOL FOR TOTAL RNA PURIFICATION

1) Transfer up to 100µl of RNA products to a 1.5-ml microcentrifuge tube (RNase-free) and add 5 volumes of RNA Binding Buffer (e.g., Add 250µl of RNA Binding Buffer to a 50µl RNA sample). Mix by shaking vigorously.

2) Add an equal volume of 70% ethanol to the sample mixture (e.g., Add 300µl of 70% ethanol to 300µl of sample mixture of step 1) and shake vigorously. Break up any precipitate with pipetting.

3) Place an RNA Binding mini spin column in a 2-ml collection tube and transfer 500µl of the sample mixture of step 2 to the column

4) Centrifuge at 14,000g-16,000g for 1 minutes. Discard the flow-through, place the column back into the collection tube and transfer the remaining sample to the same RNA Binding mini spin column.

5) Centrifuge at 14,000g-16,000g for 1 minute. Discard the flow-through and place the RNA Binding mini spin column back in the collection tube.

6) Add 600 µl of Wash Buffer* 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. **Ensure ethanol was added to Wash Buffer prior to use this kit the 1st time.*

7) Centrifuge at 14,000g-16,000g for another 3 minutes to dry the matrix of the column.

8) 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.

9) 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**
   - *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 60ºC 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.