GRS Total RNA Kit – Plant –
#GK10.0100 (100 preps)

(FOR RESEARCH ONLY)

Sample: up to 100 mg of fresh plant tissue, up to 25 mg of dry plant tissue.
Expected Yield : 5-30 µg total RNA from young leaf samples
Format : spin column
Operation Time : within 30 minutes
Elution Volume : 25-100 µl

Product Description

The GRS Total RNA Kit - Plant - provides an efficient and fast method for the purification and/or concentration of high quality total RNA (including mRNA, tRNA and rRNA) from plant tissue and cells. Optional DNAse treatment can be included in the protocol to remove undesired DNA residue. Eluted purified RNA is suitable for RT-PCR, One-step qRT-PCR, Northern Blotting, mRNA selection, cDNA synthesis, and primer extension.

Principle

Samples are ground in liquid nitrogen and subsequently filtered to remove debris. 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 30 minutes with typical RNA yields of 5-30 µg without the use of phenol extraction.

Quality Control

The quality of the GRS Total RNA Kit - Plant - is tested on a lot-to-lot basis by isolating total RNA from 25 mg young leaf sample. Quantity and Quality are ascertained by spectroscopy and gel electrophoresis.

Caution

Buffers R1 and R2 contain 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 plasticware. 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

Contents	(4 preps)	(100 preps)
Buffer R1	3 ml	60 ml
Buffer R2	3 ml	60 ml
Wash Buffer 1	2 ml	50 ml
Wash Buffer 2*	1,5 ml	25 ml + 12,5 ml
RNase-free Water	1 ml	15 ml
RNA mini spin column	4	100
Filter column	4	100
2,0-ml collection tube	8	200
1,5-ml microtube (RNase-free)	0	200
DNase I solution**	0	0,55 ml
DNase I reaction buffer **	0	5 ml

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

Notes

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

** DNase I solution and reaction buffer are shipped at room temperature and should be stored at -20ºC for up to 1 year. One should consider to prepare small aliquots, as it is recommended not to repeat thawing and freezing more than 3 times.

Note that free sample kits (4 preps) are not supplied with DNase I nor with RNase-free microtubes.

Storage

All components, except for DNase I solution and DNase I reaction buffer, should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37ºC followed by cooling to 25ºC.

DNase I treatment

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 8 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:

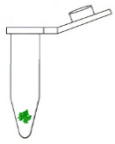
- Purified RNA (in RNase-free water): 5-40 µl
- DNase I Reaction Buffer (1x): 5 µl
- DNase I Solution : 0,5 µl for each µg of purified RNA
- RNase-free water: make up to final volume of 50 µl

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

DNase I can be removed from the reaction mixture by standard phenol extraction. Alternatively, add 250 µl of buffer R1 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 9 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 PLANT

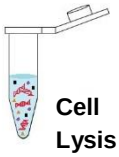
The composition of metabolites, such as polysaccharides, polyphenols, and proteins is highly dependent on the plant species and has a substantial influence on the lysis efficiency. This kit is provided with **2 different lysis buffers (R1 and R2)**. The standard protocol uses Buffer R1, whereas Buffer R2 contains an additional detergent suitable for plant samples with high polysaccharide content.



1) Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue, grind in liquid nitrogen and transfer the powder to a 1,5-ml microcentrifuge tube (RNase-free).

2) Add **500µl of Buffer R1 or Buffer R2 (see above)** and 5 µl of β-mercaptoethanol and mix by vortexing.

3) Incubate **at 60ºC** for 5 minutes.



Cell Lysis

4) Place a **Filter Column** in a 2-ml collection tube and transfer the sample mixture to the Filter Column. Centrifuge at 1.000g for 1 minute. Discard the Filter Column.

5) Add 250 µl of absolute ethanol to the filtrate and mix well by shaking vigorously.

6) Place the **RNA mini spin column** in a 2-ml collection tube and transfer the sample mixture to the column.

RNA Binding

7) 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 collection tube containing the flow-through and place the RNA mini spin column in a new collection tube.

8) *[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 centre of each spin column. Incubate at **room temperature** for **10-15 minutes**.

Wash

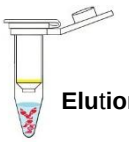
9) Add **400 µl** of **Wash Buffer 1** and centrifuge at **14.000g-16.000g** for **30 seconds.** Discard the flow-through and place the RNA mini spin column back in the collection tube. Add **600 µl** of **Wash Buffer 2*** and centrifuge at **14.000g-16.000g** for **30 seconds**

10) Discard the flow-through and add **600 µl** of **Wash Buffer 2*** and centrifuge at **14.000g-16.000g** for **30 seconds.**

Wash

11) 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.

12) 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 centre 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.



Elution

13) 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.

***check if ethanol is added first time the kit is used; see Notes on page 2.**

TROUBLESHOOTING

1. **Low Yield**
    - *Clogged Column*
        - **i.** Reduce the amount of sample material
        - **ii.** Insufficient disruption and/or homogenization
        - **iii.** Centrifugation temperature too low (should be done at 20ºC-25ºC)
    - *Incorrect RNA Elution Step*
        - **i.** Ensure that the RNase-free water is completely adsorbed after being added to the centre 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 RNAse (which can be detected by gel analysis), may be the result of improper handling of starting material

ORDERING INFORMATION – GRS Nucleic Acid Purification Kits

Reference #	Product Name	Quantity (kit)
GK01.0100	GRS PCR & Gel Band Purification Kit	100 preps
GK02.0100	GRS Genomic DNA Kit - Blood & Cultured Cells	100 preps
GK03.0100	GRS Genomic DNA Kit – Tissue	100 preps
GK04.0100	GRS Genomic DNA Kit – Plant	100 preps
GK05.0100	GRS Pure DNA Kit	100 preps
GK06.0100	GRS Genomic DNA Kit – BroadRange	100 preps
GK07.0100	GRS Genomic DNA Kit – Bacteria	100 preps
GK08.0100	GRS Total RNA Kit - Blood & Cultured Cells	100 preps
GK09.0100	GRS Total RNA Kit – Tissue	100 preps
GK10.0100	GRS Total RNA Kit – Plant	100 preps
GK11.0050	GRS microRNA Kit	50 preps
GK12.0050	GRS Viral DNA/RNA Purification Kit	50 preps
GK13.0100	GRS Plasmid Purification Kit	100 preps
GK15.0100	GRS Pure RNA Kit	100 preps
GK16.0100	GRS Total RNA Kit – Bacteria	100 preps
GK17.0100	GRS Total RNA Kit – Yeast & Fungus	100 preps
GK23.0100	TripleXtractor directRNA Kit	100 preps
GK25.0100	GRS Genomic DNA Kit – Card	100 preps
GK26.0050	GRS FullSample Purification Kit	50 preps
GK73.0010	GRS Plasmid Purification Kit – Transfection Grade	10 preps
GK73.0025	GRS Plasmid Purification Kit – Transfection Grade	25 preps

Note: Individual components (buffers, columns, tubes, enzymes) can be purchased separately. For more information, please contact us via info@grisp.pt

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