GRS Genomic DNA Kit – Plant – #GK04.0100 (100 preps)

(FOR RESEARCH ONLY)

Sample :	up to 100mg of fresh plant tissue, up to 25 mg of dry plant tissue
Expected Yield :	3-5µg total DNA from 100mg *Arabidopsis thaliana* young leaf samples 20-25µg total DNA from 100mg *Nicotiana tabacum* young leaf samples
Format :	spin column
Operation Time :	within 60 minutes
Elution Volume :	100µl

Product Description

The GRS Genomic DNA Kit - Plant - provides an efficient and fast method for the purification and or concentration of high quality total DNA (including genomic DNA, mitochondrial DNA, and chloroplast DNA) from plant tissue and cells. Eluted purified DNA is suitable and ready-to-use for PCR, real-time PCR, Southern Blotting and RFLP.

Principle

Samples are ground in liquid nitrogen and further disrupted by incubation in lysis buffer. The lysate is treated with RNase A to degrade RNA and subsequently filtered to remove cell debris and other precipitates. The buffer system is optimized to allow selective binding of DNA 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 DNA is subsequently eluted with a low salt Elution Buffer (or TE). The entire procedure can be completed within 60 minutes without phenol extraction or ethanol precipitation with typical DNA yields of up to 50µg.

Quality Control

The quality of the GRS Genomic DNA Kit - Plant - is tested on a lot-to-lot basis by isolating total DNA from 50mg young leaf sample. Quantity (more than 10 µg) and Quality are ascertained by spectroscopy and gel electrophoresis.

Caution

Some Buffers contain chaotropic salt which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

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

Kit Contents (100 preps)

Buffer PL1A	50 ml
Buffer PL1B	50 ml
Buffer PL2	15 ml
Buffer PL3*	30 ml
Wash Buffer 1	45 ml
Wash Buffer 2**	25 ml
Elution Buffer	30 ml
RNase A (10mg/ml)	0,55 ml
Genomic DNA Mini Spin Column	100
Filter Column	100
2,0-ml collection tube	200
1,5-ml microtube (DNase/RNase free)	200

Required Components (not included)

Ethanol (96%-100%)
Centrifuge for microtubes
Isopropanol
Pipets (and tips)
Vortex
Water bath or Thermoblock

Notes

Preparation

* Add 60 ml of isopropanol [not included] to Buffer PL3 prior to initial use. After isopropanol has been added, mark the bottle to indicate that this step has been completed.
** Add 100 ml ethanol (96%-100%) [not included] to Wash Buffer 2 prior to initial use. After ethanol has been added, mark the bottle to indicate that this step has been completed.

Pigment Removal

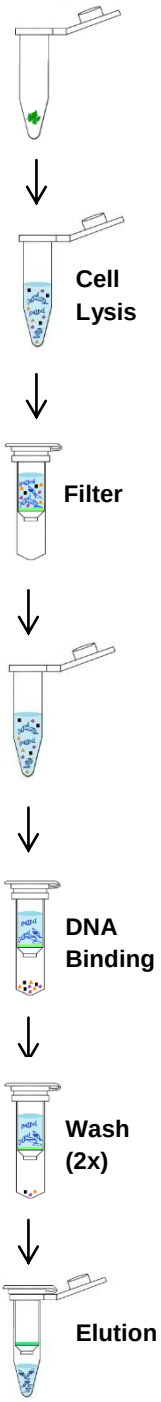
If a few pigments remain on the column after washing with Wash Buffer 2, one could perform the following optional procedure prior to DNA elution: add 400µl of 100% ethanol and centrifuge at maximum speed for 30 seconds. Discard the flow-through and place the column back in the collection tube and centrifuge again for another 3 minutes at 14.000g-16.000g to dry the matrix of the column.

Storage

RNase A (10mg/ml) should be stored at -20ºC. 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 37ºC followed by cooling to 25ºC.

PROTOCOL FOR TOTAL DNA 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 (PL1A** and **PL1B)**. The standard protocol uses Buffer PL1A, whereas Buffer PL1B contains an additional detergent suitable for plant samples with high polysaccharide content.



1) Cut off 50mg (up to 100mg) of fresh or frozen plant tissue (or 10-25mg of dried sample), grind in liquid nitrogen and transfer the powder to a 1,5-ml microcentrifuge tube.

2) Add **400µl of Buffer PL1A or Buffer PL1B (see above)** and **5µl of RNase A** and mix by vortexing (Do not mix Buffer PL1A or PL1B and RNase A prior to use).

3) Incubate **at 65ºC** for 10 minutes. Invert occasionally (Preheat Elution Buffer for step 12).

4) Add **100µl of Buffer PL2**, mix by vortexing and incubate on ice for 3 minutes.

5) 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.

6) Transfer the flow-through into a new 1,5-ml microcentrifuge tube. Add **1,5 volumes of Buffer PL3*** to the lysate and vortex for a 5-10 seconds (for example: 750µl PL3 to 500µl of lysate). **(*check if isopropanol is added first time the kit is used, see Notes on page 2)**

7) Place the **Genomic DNA Mini Spin Column** in a 2-ml collection tube and transfer 700µl of the sample mixture (including any precipitates if present) to the column.

8) Centrifuge at 14.000g-16.000g for 2 minutes. Discard the flow-through from the collection tube and place the column back in the same collection tube. Add the remaining sample mixture from step 6 and centrifuge again for 2 minutes. Discard the flow-through from the collection tube and place the column back in the same collection tube.

9) Add **400µl of Wash Buffer 1** and centrifuge at 14.000g-16.000g for 30 seconds. Discard the flow-through and place the column back in the collection tube. Add **600µl of Wash Buffer 2*** and centrifuge at 14.000g-16.000g for 30 seconds **(*check if ethanol is added first time the kit is used; see Notes on page 2)**.

10) Discard the flow-through, place the column back in the collection tube, and centrifuge for another 3 minutes at maximum speed to dry the matrix of the column.

11) [Optional Step] For the removal of any remaining pigment: see Notes on page 2

12) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet **100µl of Elution Buffer (preheated at 65ºC)** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3-5 minutes.
Notes: Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100µl. To increase concentration elute with 30-50µl. To increase yield, elute with 200µl.

13) Centrifuge for 30 seconds at 14.000g-16.000g to elute purified total DNA. Discard the spin column and use DNA immediately or store at -20ºC.

TROUBLESHOOTING

1. **Low Yield**
   - *Clogged Column*
     - **i.** Reduce the amount of sample material
     - **ii.** Insufficient disruption and/or homogenization
   - *Precipitate was formed at DNA Binding Step*
     - **i.** Reduce the amount of sample material
     - **ii.** Prior to loading the column, break up precipitate in ethanol-added lysate
   - *Incorrect DNA Elution Step*
     - **i.** Ensure that the Elution Buffer is completely adsorbed after being added to the centre of the spin column
   - *Incomplete DNA Elution*
     - **i.** Elute twice to increase overall yield
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.** DNA denaturation/fragmentation (which can be detected by gel analysis), may be the result of improper/long storage of samples.

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

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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