GRS Genomic DNA Kit – Plant

#GK04.0100 (100 preps) | GK04s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	up to 100mg of fresh plant tissue, up to 25mg of dry plant tissue
Expected Yield :	depends on type and part of plant 3-5µg total DNA from 100mg *Arabidopsis thaliana* (Thale Cress) young leaf samples 20-25µg total DNA from 100mg *Nicotiana tabacum* (Tobacco) young leaf samples
Format :	spin column
Operation Time :	within 60 minutes
Elution Volume :	30-200µl

Product: 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.

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 5-20 µg (up to 50µg).

QC: 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 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	GRS (100 preps)	sample (4 preps)	LITE* (200 preps)
Buffer PL1A	50 ml	2 ml	100 ml
Buffer PL1B	50 ml	2 ml	100 ml
Buffer PL2	15 ml	1 ml	30 ml
Buffer PL3***	30 ml	1.5 ml	60 ml
Wash Buffer 1	45 ml	2 ml	90 ml
Wash Buffer 2**	25 ml	1 ml	50 ml
Elution Buffer	30 ml	1 ml	60ml
RNase A (10mg/ml)	0.55ml	25μl	1.10ml
Genomic DNA mini spin column	100	4	200
Filter column	100	4	200
1.5-ml microtube (DNase/RNase free)	200	-	-
2-ml collection tube	200	8	400

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock
Isopropanol (2-propanol)
Liquid Nitrogen

*LITE versions of GRS NAP Kits contain only the necessary buffers and columns for the standard protocols. Additional components can be purchased separately.
** Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottle/tube, prior to initial use. After Ethanol has been added, mark the bottle/tube to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.
***Add isopropanol [not included] to Buffer PL3, as indicated on the bottle/tube, prior to initial use.
After isopropanol has been added, mark the bottle/tube to indicate that this step has been completed.

Storage: RNase A is a stable enzymes and transport is carried out either with or without cooling. Upon arrival, 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. Store for up to 2 years.

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.

PROTOCOL FOR 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 5-10 seconds (for example: 750µl PL3 to 500µl of lysate). (*Ensure isopropanol was added 1st time prior to use)
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 (*Ensure ethanol was added 1st time prior to use).
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: page 2)
12. Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipette 100µl of Elution Buffer (preheated at 65ºC) directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 minutes.
    Notes: 1) Instead of Elution Buffer, DNA can also be diluted with TE or water; pH ideally should be 8.0-8.5. 2) 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 center 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.