revised: 30-07-2012
printed: 30-07-2012

GRS Pure DNA Kit
#GK05.0100

(FOR RESEARCH ONLY)

Sample :	up to 100µl of DNA products
Expected Yield :	up to 90%
Format :	spin column
Operation Time :	20 minutes
Elution Volume :	20-50 µl

Product Description

The GRS Pure DNA Kit provides an efficient and fast method for the purification and or concentration of high quality DNA (50bp to 30kb) from samples containing partial purified DNA (genomic DNA, mitochondrial DNA, PCR products, etc) obtained via other DNA isolation methods. The purified DNA is suitable for all common downstream applications including PCR, RFLP, cloning, library construction, Southern blot analysis, and DNA sequencing.

Principle

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

Quality Control

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

Caution

The DNA Binding Buffer contains guanidine thiocyanate 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)

DNA Binding Buffer	80 ml
Wash Buffer *	25 ml
Elution Buffer	6 ml
DNA Binding mini spin Column	100
2,0-ml collection tube	100
1,5-ml microtube (DNAse/RNAse free)	100

Required Components (not included)

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

Notes

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

Storage

All 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 DNA PURIFICATION

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

2) Place the **DNA Binding mini spin column** in a 2,0-ml collection tube and transfer the sample mixture of step 1 to the column.

3) Centrifuge at 14.000g-16.000g for 30 seconds.

4) Discard the the flow-through, and place the spin column back in the collection tube.

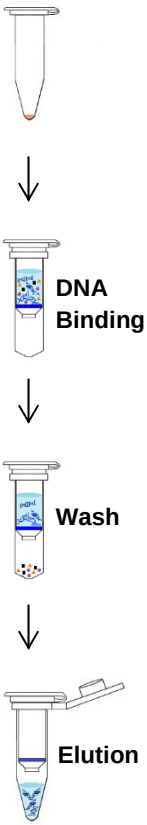
5) Add 600µl of **Wash Buffer*** and let stand for 1 minute.
*(check if ethanol is added first time the kit is used; see notes on page 2)

6) Centrifuge for 14.000g-16.000g for 30 seconds and discard the flow-through.

7) Place the 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.

8) Transfer the spin column to a new 1,5-ml microcentrifuge tube (DNAse Free) and pipet 20µl-50µl **Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 2 minutes.
Notes: Yield could be increased using pre-warmed Elution Buffer (60ºC). Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5.

9) Centrifuge for 2 minutes at 14.000g-16.000g to elute purified DNA. Discard the spin column and use DNA immediately or store at -20ºC.



TROUBLESHOOTING

1. **Low Yield**
   - *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. For large DNA fragments (>10kb), using preheated (60ºC-70ºC) elution buffer may improve the elution efficiency
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. In case of DNA denaturation (which can be detected by gel analysis), incubate eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the DNA.

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
GK11.0100	GRS microRNA Kit	100 preps
GK12.0100	GRS Viral DNA/RNA Purification Kit	100 preps
GK13.0100	GRS Plasmid Purification Kit	100 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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