GRISP RESEARCH SOLUTIONS

GRS Pure DNA Kit

#GK05.0100 (100 preps) | GK05s (trial size, 4 preps)
(FOR RESEARCH ONLY)

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

Product: 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.

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%.

QC: 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.

Kit Contents

	(100 preps)	(4 preps)
DNA Binding Buffer	80 ml	3 ml
Wash Buffer*	25 ml	1 ml
Elution Buffer	6 ml	1 ml
DNA Binding mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	100	-
2-ml collection tube	100	4

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

* 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.

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.

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. Store for up to 2 years.

References:

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

PROTOCOL FOR DNA PURIFICATION

1. Transfer up to 100 µl of DNA products to a 1.5-ml microcentrifuge tube (DNase-free) and add 5 volumes of DNA Binding Buffer. (e.g., Add 250 µl of DNA Binding Buffer to a 50 µl DNA sample). Mix by shaking vigorously.
2. Place the DNA Binding mini spin column in a 2-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 flow-through, and place the spin column back in the collection tube.
5. Add 600µl of Wash Buffer* and let stand for 1 minute.
   **Ensure ethanol was added 1st time prior to use*
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-mlmicrocentrifuge tube (DNase-free) and pipet 20µl-50µl Elution Buffer directly to the center 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 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. In case of DNA denaturation, incubate eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the DNA.