GRS Genomic DNA Kit – Tissue – #GK03.0100 (100 preps)

(FOR RESEARCH ONLY)

Sample :	up to 30mg of tissue or 25mg of paraffin-embedded tissue
Expected Yield :	up to 50µg DNA
Format :	spin column
Operation Time :	within 30 minutes (60 min for FFPE)
Elution Volume :	30-200 µl

Product Description

The GRS Genomic DNA Kit – Tissue - provides an efficient and fast method for the purification and or concentration of high quality total DNA (including genomic DNA, mitochondrial DNA and viral DNA) from a variety of tissues, including tailsnips, liver, kidney, brain, adipose tissue, earpunches, insects, and FFPE. Eluted purified DNA (approximately 20-30kb) is suitable for PCR, and other enzymatic reactions.

Principle

The GRS Genomic DNA Kit – Tissue - uses chaotropic salts and proteinase K to lyse cells and to denature proteins. The provided micropestle improves tissue sample homogenization and therefore to reduce the time required for cell lysis. The buffer system is optimized to allow selective 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 genomic DNA is subsequently eluted by a low salt Elution Buffer or TE or water. The entire procedure can be completed within an hour without phenol/chloroform extraction or alcohol precipitation, with typical DNA yields of up to 50µg.

Quality Control

The quality of the GRS Genomic DNA Kit – Tissue - is tested on a lot-to-lot basis by isolating total DNA from 20mg of a mouse liver sample. Purified DNA is quantified using a spectrophotometer with a typical yield of more than 10µg of genomic DNA and a A260nm/A280nm ratio of 1.8-2.0. Quality is further checked by agarose gel electrophoresis.

Caution

Buffer TC1 contains guanidine hydrochloride 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 BC2	30 ml
Buffer TC1	40 ml
Wash Buffer 1	45 ml
Wash Buffer 2*	25 ml
Elution Buffer	30 ml
RNase A (10mg/ml)	0,55 ml
Proteinase K (10mg/ml)**	2 x 11mg
gDNA plus columns	100
2,0-ml collection tube	200
1,5-ml microtube (DNase/RNase free)	200
Micropestle	100

Required Components (not included)

Ethanol (96%-100%)
Centrifuge for microtubes
Pipets (and tips)
Vortex
Water bath or Thermoblock
Xylene
15-ml centrifuge tubes

Notes

* 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.
** Add 1,1ml of ddH_2O to each vial of Proteinase K and store at 4ºC.

Storage

RNase A (10mg/ml) should be stored at -20ºC and Proteinase K (10mg/ml) at 4ºC. 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 DNA PURIFICATION FROM PARAFFIN-EMBEDDED TISSUE

1) Slice small sections (**up to 25mg**) from blocks of paraffin-embedded tissue and transfer to a 1,5-ml microcentrifuge tube. Add 1 ml of **Xylene**. Shake vigorously, and incubate at **room temperature** for **10 minutes**. During incubation, invert the tube regularly.

2) Centrifuge for 3 minutes at 14.000g-16.000g and discard the supernatant, using a pipet.

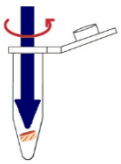
3) Add 1 ml of absolute ethanol to the pellet and mix by inverting. Centrifuge for 3 minutes at 14.000g-16.000g and discard the supernatant, using a pipet. Repeat this step twice.

4) Open the tube and air-dry (37ºC) for 15 minutes to evaporate any remaining ethanol.

5) Proceed with step 2 of the Tissue Protocol on page 3 (Addition of Buffer BC2).

PROTOCOL FOR DNA PURIFICATION FROM TISSUE

1) Cut up to 30mg of animal tissue (or 5mm of mouse tail) and transfer to a 1,5-ml microcentrifuge tube. In case of some tissues, like spleen that contain a very high number of cells, the starting material should be reduced to 10mg. Use the micropestle to grind the tissue to pulp.

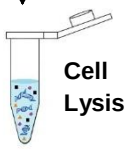


Homogenization

2) Add 200µl of **Buffer BC2** and continue to homogenize the sample by grinding.

3) Add 20µl of **Proteinase K** (10mg/ml), mix by shaking vigorously, and incubate at **60ºC** for 30 minutes. During incubation, invert the tube regularly.

4) Add 200µl of **Buffer TC1**, mix by shaking vigorously, and incubate at **60ºC** for at least 20 minutes. During incubation, invert the tube regularly. (Note that sample lysate should become clear. If there is still insoluble material present following the lysis step, centrifuge for 2 minutes at 14.000g-16.000g and transfer the supernatant to a new 1,5-ml microcentrifuge tube). [At this time, preheat the Elution Buffer in a 60ºC water bath to be used in step 11].



Cell Lysis

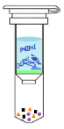
5) [*Optional step; If RNA-free DNA is required*] Allow the mixture to cool to room temperature and add 4µl of **RNase A (10mg/ml)**, mix by shaking vigorously and incubate for 5 minutes at room temperature

6) Add 200µl of absolute ethanol to the lysate and mix immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.

DNA Binding

7) Place the **gDNA plus mini spin column** in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.

8) Centrifuge at 14.000g-16.000g for 2 minutes. Discard the collection tube containing the flow-through and place the gDNA plus mini spin column in a new collection tube.



Wash

9) Add 400µl of **Wash Buffer 1** and centrifuge at 14.000g-16.000g for 30 seconds. Discard the flow-through and place the gDNA plus mini spin 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).**

Wash

10) Discard the flow-through and place the gDNA plus mini spin column back in the collection tube and centrifuge for another 3 minutes at 14.000g-16.000g to dry the matrix of the column.

Elution

11) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl **preheated Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature 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.

Centrifuge for 30 seconds at 14.000g-16.000g to elute purified genomic 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.** Prior to loading the sample, break up the precipitate in the ethanol-added lysate
   - *Incomplete tissue lysis*
     - **i.** Add additional Proteinase K and extend the incubation time in the Lysis step
     - **ii.** Following lysis step, centrifuge for 2 minutes at 14.000g-16.000g to remove debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA binding step
   - *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.** In case of protein contamination, the amount of starting material should be reduced.
     - **iii.** DNA denaturation/fragmentation (which can be detected by gel analysis), may be the result of improper/long storage of “fresh” samples. Use fresh samples, or freeze samples in liquid nitrogen immediately and store at -80ºC

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

GRiSP Research Solutions
Rua Alfredo Allen, 455|461
4200-135 Porto, PORTUGAL
Tel: +351 22 030 15 99 | Fax: +351 22 030 15 97
www.grisp.pt| info@grisp.pt