GRS Genomic DNA Kit – Card – GK25.0100 (100 preps)

(FOR RESEARCH ONLY)

Sample :	dried blood spots (on Whatman® FTA® Card)
Expected Yield :	typically more than 300ng of pure genomic DNA from 6mm dried blood spots
Format :	spin column
Operation Time :	approximately 1 hour.
Elution Volume :	30-100 µl

Product Description

The GRS Genomic DNA Kit – Card provides an easy and efficient method for the purification of high quality genomic DNA from dried blot spots (on Whatman® FTA® Cards). Eluted purified DNA (approximately 20-30kb) is suitable for PCR, real-time PCR, and other enzymatic reactions.

Principle

The GRS Genomic DNA Kit – Card uses proteinase K and chaotropic salts to lyse cells and to denature proteins. Carrier RNA is included to minimize DNA loss to plasticware. 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 300ng from a 6mm dried blot spot.

Quality Control

The quality of the GRS Genomic DNA Kit – Card is tested on a lot-to-lot basis by isolating genomic DNA from a 6mm dried blot spot on a Whatman® FTA® Card. Yields are typically at least 300ng of pure genomic DNA, as determined by spectrophotometer (A260nm/A280nm ration of 1.8-2.0) and verified by electrophoresis on a 0.8% agarose gel.

Caution

Some buffers contain harmful irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Literature

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

Kit Contents

Buffer C1	30 ml
Buffer C2	30 ml
Wash Buffer 1	45 ml
Wash Buffer 2*	25 ml
Elution Buffer	75 ml
Carrier RNA**	1 mg
Proteinase K (11mg)**	2x
Genomic DNA mini spin column	100
2,0-ml collection tube	200
1,5-ml microtube (DNAse/RNAse free)	300

Required Components (not included)

Ethanol (96%-100%)
Single hole paper punch

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.
** In order to obtain a working solution of 1 µg/µl, add 1ml of Elution Buffer to the Carrier RNA, vortex, and ensure that RNA is completely dissolved. Aliquot the Carrier RNA solution in convenient amounts in RNA-free tubes and store at -20ºC. Avoid freeze-thawing for more than three times.
*** Add 1,1ml of ultrapure water to each vial of Proteinase K, in order to obtain a Proteinase K solution of 10mg/ml. Vortex vigorously and store at +4ºC upon use.

Storage

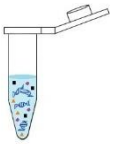
Store Carrier RNA at -20ºC and Proteinase K at +4ºCºC. All other components should be stored at room temperature (15-25ºC). Close bottle containing Wash Buffer 2 tightly to avoid ethanol evaporation.

DRIED BLOT SPOT PROTOCOL

0) **Before beginning:** Check if Ethanol was added to Wash Buffer 2, water to Proteinase K and Elution Buffer to Carrier RNA (see page 2). Then, for each sample to be processed, mix **1 µl of RNA Carrier Solution and 200 µl of Buffer C1** in a suitable RNA-se free tube. Vortex briefly and keep at hand for step 3. Preheat Elution Buffer to 60ºC to be used in step 10.

1) Using a single-hole paper punch, cut out a 6mm diameter circle from a dried blood spot (Whatman® FTA® Card) , and transfer the paper piece to a RNAse/DNAse-free 1,5ml microtube.

2) Add **200 µl of Buffer C2** and **20 µl of Proteinase K (10mg/ml)** to the paper piece, and mix well by vortexing. Ensure that the paper is completely immersed in the buffer. Lyse by incubation at 60ºC for 30 minutes. Invert the tube regularly.



3) Add **200 µl of Buffer C1 (containing RNA Carrier)** and mix well by vortexing. Incubate at 60ºC for another 20 minutes. Invert the tube regularly.

4) After incubation, centrifuge briefly and transfer the lysate to a new RNAse/DNAse-free 1,5ml microtube. Add **200µl of ethanol** (96%-100%) and mix by shaking vigorously immediately for 10 seconds. In case precipitate appears, break it up by pipetting

5) Place the **genomic DNA mini spin column** in a 2,0-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column. Centrifuge at 14.000g-16.000g for 2 minutes.

6) Discard the flow-through and place the spin column in a **new** 2,0-ml collection tube and add **400µl Wash Buffer 1**. Centrifuge for 30 seconds at 14.000g.16.000g and discard the flow-through.

7) Place the **spin column** back in the collection tube. Add **600µl** of **Wash Buffer 2** (ethanol added) Centrifuge for 30 seconds at 14.000g-16.000g and discard the flow-through.

8) Place the spin column back in the 2-ml collection tube and centrifuge for 3 minutes at 14.000g-16.000g to dry the column membrane completely and to get rid of trace amounts of ethanol. Discard flow-through and collection tube.

9) Remove the spin column carefully and place into a new 1,5-ml microtube.

10) Pipet **100µl Elution Buffer (pre-heated)** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3 minutes.
Notes: Instead of Elution Buffer, DNA can also be diluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100µl. Concentration can be increased by using less volume (30µl-50µl) or alternatively yield can be increased by using more volume (200µl).

11) Centrifuge for 1 minute 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**
   - *Incomplete Lysis*
     i. Ensure that dried blood spot sample is completely immersed in the buffer. Verify that Carrier RNA was added to Buffer C1.
   - *Incomplete DNA elution*
     i. If using water to elute the DNA, make sure that the pH is above 7,5. Water should be prepared freshly as CO_2 from the environment could cause acidification and lower the DNA´s solubility.

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

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