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GRS Genomic DNA Kit – Card

#GK25.0100 (100 preps) | GK25s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	dried blood spots (on Whatman® FTA® Card)
Expected Yield :	typically 150ng to 300ng of pure genomic DNA from 6mm dried blood spots
Format :	spin column
Operation Time :	approximately 1 hour
Elution Volume :	30-100µl

Product: 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.

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 150ng to 300ng from a 6mm dried blot spot.

QC: 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 200ng of pure genomic DNA, as determined by spectrophotometer (A260nm/A280nm ratio of 1.8-2.0) and verified by electrophoresis on a 0.8% agarose gel.

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

15 YEARS 2008-2023

Kit Contents	(100 preps)	(4 preps)
Buffer C1	30 ml	3 ml
Buffer C2	30 ml	4 ml
Wash Buffer 1	45 ml	2 ml
Wash Buffer 2*	25 ml	1 ml
Elution Buffer	75 ml	6 ml
Carrier RNA***	1mg	1mg
Proteinase K**	2x 11mg	1mg
Genomic DNA mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	200	-
2-ml collection tube	200	8

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

* 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 Water (ultrapure) [not included] to Proteinase K, as indicated on the tube, prior to initial use.
After Water has been added, mark the tube to indicate that this step has been completed.

***Add 1 ml of Elution Buffer to the Carrier RNA, as indicated on the bottle/tube, prior to initial use.
After Elution Buffer has been added, vortex and ensure that RNA is completely dissolved. Aliquot the Carrier RNA in convenient amounts in RNase-free microtubes and store at -20ºC. Avoid freeze-thawing for more than 3 times.

Storage: Store Carrier RNA at -20ºC. Proteinase K is a stable enzyme and transport is carried out either with or without cooling. Upon arrival, proteinase K (powder) should be stored at 4ºC. Once water has been added, it is recommended to store the Proteinase K solution 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.

PRIOR TO USE

Ensure ethanol was added to Wash Buffer 2, water to Proteinase K and Elution Buffer to Carrier RNA Then, for each sample to be processed, mix 1µl of RNA Carrier Solution and 200µl of Buffer C1 in a suitable RNase-free tube. Vortex briefly and keep at hand for step 3. Preheat Elution Buffer to 60ºC to be used in step 9.

DRIED BLOOD SPOT PROTOCOL

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 200µl 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 200µl 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

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5. Place the genomic DNA mini spin column in a 2-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-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 center of the spin column without touching the membrane. Incubate at room temperature for 3 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.

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.
        - **ii.** Verify that Carrier RNA was added to Buffer C1
    - *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.