GRS Genomic DNA Kit – Card

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

PRIOR TO USE:

Check if 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 1 µl of RNA Carrier Solution and 200 µl of Buffer C1 in a suitable RNase-free tube. Vortex briefly and keep at hand for step 2. Preheat Elution Buffer to 60ºC.

SUGGESTED PROTOCOL FOR DNA PURIFICATION FROM SALIVA

1. Transfer 1-100µl of Urine to a RNase/DNase-free 1.5ml microtube and centrifuge at 6,000g for 2 minutes. Discard the supernatant and add 500 µl of Elution Buffer to the pellet and vortex for 5 seconds.
   (For larger volumes: transfer urine to a 15-ml tube and centrifuge at 6,000g for 2 minutes. Discard the supernatant and add 500µl of Elution Buffer. Vortex for 5 seconds and transfer to a 1.5ml microcentrifuge tube)

2. Centrifuge at 6,000g for 2 minutes, discard the supernatant and add 200 µl of Buffer C2 and 20 µl of Proteinase K (10mg/ml), and mix well by vortexing. Lyse by incubation at 60ºC for 30 minutes. Invert the tube regularly. *Note: to increase DNA yield from male urine samples, containing sperm cells, add 20µl of 1M DTT (not included) prior to lysis.*

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-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 of Wash Buffer 1. Centrifuge at 14,000g-16,000g for 30 seconds and discard the flow-through.

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

8. Place the spin column back in the 2-ml collection tube and centrifuge at 14,000g-16,000g for 3 minutes 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 50µl Elution Buffer (pre-heated) directly to the center 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 50µl. Concentration can be increased by using less volume (30µl) or alternatively yield can be increased by using more volume (100µl).

11. Centrifuge for at 14,000g-16,000g for 1 minute to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20ºC.