GRS Genomic DNA Kit – Bacteria

#GK07.0100 (100 preps) | GK07s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : 0.5-2.0 ml of bacterial culture with up to 1x10^9 cells (Gram(+) or Gram(-))
Expected Yield : 25-30µg DNA (1x10^9 of *Escherichia coli*); 10-15µg DNA (1x10^9 of *Bacillus subtilis*)
Format : spin column
Operation Time : within 60 minutes
Elution Volume : 30-200µl

Product: The GRS Genomic DNA Kit – Bacteria – provides an efficient and fast method for the purification of high quality genomic (and viral) DNA from Gram-positive and Gram-negative bacteria, suitable for all common downstream applications such as PCR, enzymatic restriction digestion, cloning, Southern blot analysis, etc.

The GRS Genomic DNA Kit – Bacteria – can be used for both Gram (-) and Gram(+) bacterial cells. The provided Buffer G+, once supplemented with lysozyme, will lyse bacterial cell walls consisting of peptidoglycan. Proteinase K and Chaotropic salt are then used for further cell lysis and protein degradation. 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, secondary metabolites, and enzyme inhibitors are completely removed using Wash Buffer. The purified genomic DNA is subsequently eluted by a low salt Elution Buffer or TE or water. The entire procedure can be completed in less than 60 minutes without phenol/chloroform extraction or alcohol precipitation, with a typical DNA yield of 25-30µg from 1.5 ml of Escherichia coli (~10^9 cells).

QC: The quality of the GRS Genomic DNA Kit – Bacteria – is tested on a lot-to-lot basis by isolating genomic DNA from Escherichia coli (~1x10^9 cells). The purified DNA (25-30µg with a A260/A280 ratio of 1.6-1.8) is quantified with a spectrophotometer and checked by electrophoresis.

Caution: Buffer BL 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	GRS (100 preps)	sample (4 preps)	LITE* (200 preps)	Required Components (not included)
Buffer G+****	30 ml	2 ml	60 ml	Ethanol (96%-100%)
Buffer GN	30 ml	1.5 ml	60 ml	Centrifuge for microtubes
Buffer BL	40 ml	2 ml	80 ml	Pipets and tips
Wash Buffer 1	45 ml	2 ml	90 ml	Vortex
Wash Buffer 2**	25 ml	1 ml	50 ml	Water bath or Thermoblock
Elution Buffer	30 ml	1 ml	60 ml	15-ml centrifuge tubes
RNase A (10mg/ml)	0.55ml	-	-	
Proteinase K***	2x 11 mg	1 mg	4x 11 mg	
Lysozyme	110 mg	8 mg	220 mg	
Genomic DNA mini spin column	100	4	200	
1.5-ml microtube (DNase/RNase free)	200	-	-	
2-ml collection tube	200	8	400	

*LITE versions of GRS NAP Kits contain only the necessary buffers and columns for the standard protocols. Additional components can be purchased separately.

*Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottle, prior to initial use. After Ethanol has been added, mark the bottle 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 Lysozyme to Buffer G+ immediately prior to use as described in step 1 of the Gram-positive protocol on page 2. Once lysozyme has been mixed with Buffer G+, the solution can be stored for up to 1 week at +4°C.

Storage: RNase A, Lysozyme and Proteinase K are stable enzymes and transport is carried out either with or without cooling. Upon arrival, RNase A (10mg/ml) and Lysozyme (powder) should be stored at -20°C and proteinase K (powder) at 4°C. Once water has been added, it is recommended to store the Proteinase K solution at -20°C. 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 37°C followed by cooling to 25°C. Store for up to 2 years.

PROTOCOL FOR DNA PURIFICATION FROM GRAM-POSITIVE BACTERIA

1) (Pre-preparation) For each sample, transfer 200 µl Buffer G+ to a 15-ml centrifuge tube and add 0.8 mg Lysozyme to a final concentration of 4 mg/ml. Vortex until the Lysozyme is completely dissolved.

2) Transfer up to $1x10^9$ bacterial cells to a 1.5-ml microtube and centrifuge at 14,000g-16,000g for 1 minute. Discard Supernatant.

3) Add 200 µl of Buffer G+ supplemented with Lysozyme, and resuspend the pellet immediately by pipetting or thoroughly vortexing. Incubate at 37°C for 30 minutes. During incubation, invert the tube regularly. Add 20 µl of Proteinase K solution and incubate at 60°C for 15 minutes. Invert the tube regularly.

4) Proceed with step 3 of the protocol for the DNA purification from Gram-negative bacteria (page 3).

PROTOCOL FOR DNA PURIFICATION FROM GRAM-NEGATIVE BACTERIA

1) Transfer up to 1x10^9 bacterial cells to a 1.5-ml microtube and centrifuge at 14,000g-16,000g for 1 minute. Discard Supernatant.

2) Add 180 µl of Buffer GN and resuspend the pellet immediately by pipetting or thoroughly vortexing. Add 20 µl of Proteinase K solution and incubate at 60ºC for 15 minutes. Invert the tube regularly [At this time, preheat the Elution Buffer in a 70ºC water bath to be used in step 10].

3) **[optional; when RNA-free DNA is required]** Allow the mixture to cool to room temperature and add 5µl of RNase A (10mg/ml), vortex, and incubate for 5 minutes at room temperature.

4) Add 200µl of absolute ethanol to the lysate and mix immediately for 10 seconds by shaking vigorously. 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.

6) Centrifuge at 14,000g-16,000g for 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.

7) Add 400µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the genomic DNA mini spin column back in the collection tube.

8) Add 600µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds (*Ensure ethanol was added 1st time prior to use).

9) Discard the flow-through and place the genomic DNA 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.

10) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 100µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 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 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
    - *Precipitate was formed at DNA Binding Step*
        - **i.** Reduce the amount of sample material
        - **ii.** Prior to loading the column, break up precipitate in ethanol-added lysate
    - *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 60°C for 5 minutes in order to evaporate ethanol.