GRS Genomic DNA Kit – Bacteria – #GK07.0100 (100 preps)

(FOR RESEARCH ONLY)

Sample :	0,5-2,0 ml of bacterial culture with up to 1x10^9 cells (Gram (+) or Gram (-))
Expected Yield :	25-30 µg (1x10^9 of *Escherichia coli*); 10-15 µg (1x10^9 of *Bacillus subtilis*)
Format :	spin column
Operation Time :	less than 1 hour
Elution Volume :	30-200 µl

Product Description

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.

Principle

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 25-30 µg from 1, 5 ml of *Escherichia coli* (~10^9 cells).

Quality Control

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 µg-30 µg with an 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

Contents	(100 preps)
Buffer G+*	30 ml
Buffer GN	30 ml
Buffer BL	40 ml
Elution Buffer	30 ml
Wash Buffer 1	45 ml
Wash Buffer 2**	25 ml
Genomic DNA mini spin column	100
2,0-ml collection tube	200
1,5-ml microtube (DNase-free)	200
Lysozyme*	110 mg
Proteinase K***	2x 11 mg
RNase A (10mg/ml)	0,55 ml

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets (and tips)
Vortex
Water bath or Thermoblock
15-ml centrifuge tube (Gram (+) bacteria only)

Notes

* 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 the Buffer G+, the solution can be stored for up to 1 week at +4°C

**Add ethanol (96%-100%) [not included] to Wash Buffer 2 prior to initial use as indicated on the bottle(s). After ethanol has been added, mark the bottle(s) to indicate that this step has been completed.

***Reconstitute Proteinase K by adding DNase-free ddH_2O (volume as indicated on vial(s)) and store at +4ºC.

Storage

RNase A (10mg/ml) should be stored at -20ºC. All other components should be stored at room temperature, except proteinase K once reconstituted (see above). 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 TOTAL 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 37ºC for 30 minutes. During incubation, invert the tube regularly. Add **20 µl of Proteinase K solution** and incubate at 60ºC for 15 minutes. Invert the tube regularly. Proceed with **step 3** of the protocol for the DNA purification from Gram-negative bacteria (page 3).

PROTOCOL FOR TOTAL 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.

3) Add **200 µl of Buffer BL** and mix immediately by shaking vigorously for 10 seconds. Incubate at 70°C for at least 10 minutes (ensure the sample lysate is clear). During incubation, invert the tube regularly. [At this time, preheat the Elution Buffer in a 70ºC water bath to be used in step 11].

4) [**OPTIONAL**] If RNA-free genomic DNA is required, allow to cool to room temperature and add 5 µl **RNase A** (10mg/ml) and incubate for 15 minutes at room temperature.

5) Add **200 µl of ethanol** (96%-100%) and mix by shaking vigorously immediately for 10 seconds. In case precipitate appears, break it up by pipetting

6) 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.

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

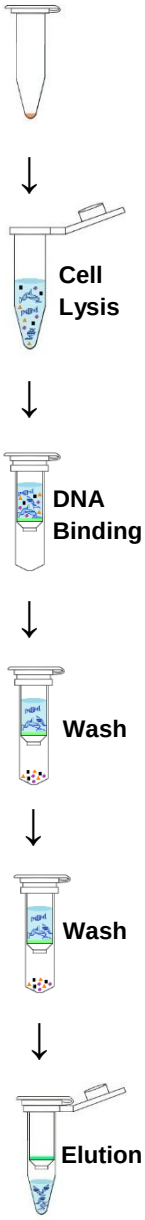
8) Place the **spin column** back in the collection tube and add **600 µl** of **Wash Buffer 2** (check if ethanol is added first time the kit is used; see notes on page 2). Centrifuge for 30 seconds at 14.000g-16.000g and discard the flow-through.

9) 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.

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

11) Pipet **100 µl Elution Buffer** directly to the centre of the spin column without touching the membrane. Incubate at room temperature for 3 minutes.
Notes: Yield could be increased using pre-warmed Elution Buffer (60-70ºC). 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).

12) 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**
   - *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 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.

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
Edifício Inovar & Crescer
4200-135 Porto, PORTUGAL
Tel: +351 22 030 15 99 | Fax: +351 22 030 15 97
www.grisp.pt| info@grisp.pt