GenElute™ Bacterial Genomic DNA Kit Protocol (NA2100, NA2110, NA2120)

Product Description

Sigma's GenElute™ Bacterial Genomic DNA Kit provides a simple and convenient way to isolate pure DNA from a variety of cultured bacteria. The kit contains all of the reagents needed to isolate and purify genomic DNA from gram-negative bacteria. For most gram-positive bacteria, the kit must be used in conjunction with the optional lysozyme (**L4919**), to effectively lyse the thick peptidoglycan cell walls. A Gram-Positive Lysis Solution is provided as a diluent for preparing the lysozyme stock solutions.

The GenElute kit combines the advantages of a silica-based system with a microspin format and eliminates the need for expensive resins, alcohol precipitation, and hazardous organic compounds such as phenol and chloroform. The bacteria are lysed in a chaotropic salt-containing solution to ensure the thorough denaturation of macromolecules. The addition of ethanol causes the DNA to bind when the lysate is spun through a silica membrane into a microcentrifuge tube. After washing to remove the contaminants, the DNA is eluted in 200 µL of a Tris-EDTA solution.

The expected yield of genomic DNA will vary depending on the cell density of the bacterial culture and the bacterial species and strain used. Appendix 2 lists the typical yield of genomic DNA purified from some gram-negative and gram-positive bacteria. DNA purified with the GenElute kit has an A_{260}/A_{280} ratio between 1.6 and 1.9 and can be up to 50 kb in length. This DNA is ready for downstream applications such as restriction endonuclease digestions, PCR, and Southern blots.

Reagents Provided	Catalog Number	NA2100 10 Preps	NA2110 70 Preps	NA2120 350 Preps
Gram-Positive Lysis Solution	L7539	3 mL	20 mL	90 mL
Lysis Solution T	B6678	2.5 mL	20 mL	90 mL
Lysis Solution C	B8803	2.5 mL	20 mL	90 mL
Wash Solution 1	**W0263**	7 mL	50 mL	225 mL
Wash Solution Concentrate	B6553	2.5 mL	20 mL	90 mL
Elution Solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0)	B6803	5 mL	35 mL	180 mL
Column Preparation Solution	**C2112**	7 mL	60 mL	225 mL
Proteinase K	**P2308**	1 x 5 mg	3 x 10 mg	2 x 100 mg
RNase A Solution	**R6148**	0.25 mL	1.7 mL	8 mL
GenElute Nucleic Acid Binding Columns in tube	C9471	10 each	70 each	5 x 70 each
Collection Tubes, 2.0 mL capacity	T5449 or T7813	3 x 10 each	3 x 70 each	15 x 70 each

Equipment and Reagents Required But Not Provided

- 37 °C water bath or heating block
- 55 °C water bath or heating block
- Pipette tips (aerosol barrier recommended)
- 1.5 mL microcentrifuge tube for lysis
- Microcentrifuge (2 mL tube, rotor equipped)*
- Ethanol (95%–100%), Catalog Nos. **E7023**, **E7148**, or **459836**
- Molecular Biology Reagent Water, Catalog No. **W4502**
- Lysozyme, Catalog No. **L4919** (for gram-positives only)
- Mutanolysin, Catalog No. **M9901** (for *Streptococcus* species only)
- Lysostaphin, Catalog No. **L7386** (for *Staphylococcus* only)

Note: To ensure proper fit of all tubes, a 24-place rotor is recommended. If you are using a 36-place rotor, we recommend using every other place for proper tube fit.

Storage and Stability

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and allow to cool to room temperature before use.

Preparation Instructions

1. **Preheat a Water Bath or Heating Block to 55 °C**

2. **Thoroughly Mix Reagents**
   Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and allow cooling to room temperature before use.

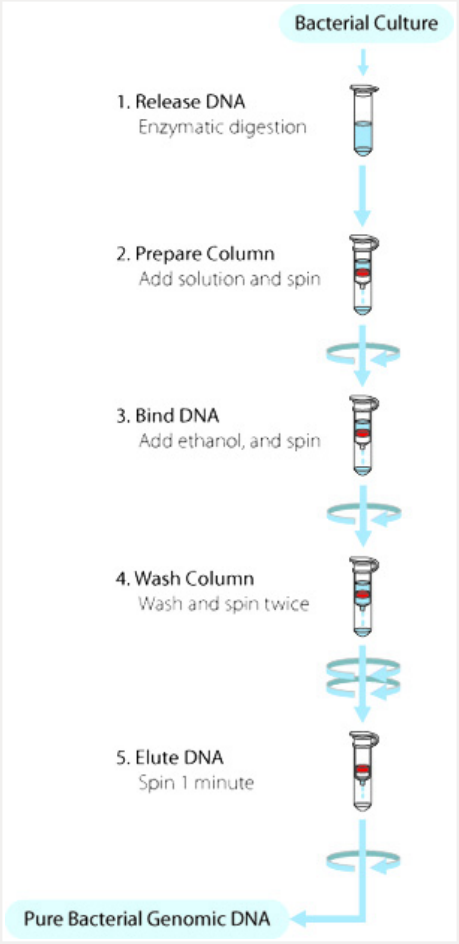
3. **Dilute Prewash Solution**
   Dilute the concentrate with 5.5 mL (10 prep package), 27.5 mL (70 prep package), or 110 mL (350 prep package) of 95–100% ethanol. After each use, tightly cap the diluted Prewash Solution to prevent the evaporation of ethanol.

4. **Dilute Wash Solution Concentrate**
   Dilute the concentrate with 10 mL (10 prep package), 80 mL (70 prep package), or 360 mL (350 prep package) of 95–100% ethanol. After each use, tightly cap the diluted Wash Solution to prevent the evaporation of ethanol.

5. **Dissolve the Proteinase K**
   Dissolve the powder in one bottle of Proteinase K in water to obtain a 20 mg/mL stock solution, according to Table 1. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature.

   Note: The Proteinase K solution must be added directly to each sample every time. Do not combine the Proteinase K and Lysis Solutions for storage.

Catalog Number	Proteinase K (mg)	Water (mL)
NA2010	10	0.5
NA2020	100	5.0



Procedure

Note: All centrifugation speeds are given in units of g. Please refer to Table 2 for information on converting g-force to rpm. If centrifuges/rotors for the required g-forces are not available, use the maximum g-force possible and increase the spin time proportionally. Spin until all liquid passes through the column

See Appendix 1 convert g-force to RPM.

Note: If minimally sheared genomic DNA is desired in downstream applications, e.g., if using the end product for long amplification PCR, mix with gentle pipetting or inversion until homogeneous instead of vortexing in the procedure that follows.

1. **Collect Blood**
   Collect whole blood in an anticoagulant tube (an EDTA tube is preferred). Whole blood should be equilibrated to room temperature before beginning preparation.

2. **Prepare Blood**
   Place 20 µL of the Proteinase K solution into a 1.5 mL microcentrifuge tube. Add up to 200 µL of the whole blood sample to the tube. For larger volumes, see Appendix. If the sample is less than 200 µL, add the Resuspension Solution to bring the volume up to 200 µL.

   Note: If the sample is already dispensed into a tube, the Proteinase K solution can be added to the sample. Vortex to ensure thorough mixing of the enzyme. Whole blood may be stored at 4 °C for at least 3 months before preparing the DNA. If residual

RNA is not a concern, continue with step 3.

Optional RNase A treatment: If RNA-free genomic DNA is required, add 20 µL of RNase A Solution and incubate for 2 minutes at room temperature; continue with step 3.

3. **Lyse Cells**
   Add 200 µL of Lysis Solution C to the sample; vortex thoroughly (15 seconds). A homogeneous mixture is essential for efficient lysis. Incubate at 55 °C for 10 minutes.

4. **Column Preparation**
   Add 500 µL of the Column Preparation Solution to each pre-assembled GenElute Miniprep Binding Column (with a red o-ring, not to be confused with other GenElute kits) and centrifuge at 12,000 x g for 1 minute. Discard the flow-through liquid.

   Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.

5. **Prepare for Binding**
   Add 200 µL of ethanol (95–100%) to the lysate from step 3; mix thoroughly by vortexing for 5–10 seconds. A homogeneous solution is essential.

6. **Load Lysate**
   Transfer the entire contents of the tube into the treated column from step 4. Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column. Centrifuge at ≥6500 x g for 1 minute. Discard the collection tube containing the flow-through liquid and place the column in a new 2 mL collection tube.

7. **First Wash**
   Prior to first use, dilute both the Prewash Solution Concentrate and the Wash Solution Concentrate with ethanol as described in the Preparation Instructions. Add 500 µL of either Prewash Solution or Wash Solution to the column and centrifuge for 1 minute at ≥6500 x g. Discard the collection tube containing the flow-through liquid and place the column in a new 2 mL collection tube.

   Note: If the whole blood sample is aged (older than 24 hours), the Prewash Solution is helpful in removing contaminants associated with older whole blood samples. If the sample is fresh, the Prewash Solution is not always necessary for the first wash.

8. **Second Wash**
   Add 500 µL of Wash Solution to the column; centrifuge for 3 minutes at maximum speed (12,000–16,000 x g) to dry the column. The column must be free of ethanol before eluting the DNA. Centrifuge the column for 1 additional minute at maximum speed if residual ethanol is observed. You may empty and re-use the collection tube if you need this additional centrifugation step. Discard the collection tube containing the flow-through liquid and place the column in a new 2 mL collection tube.

9. **Elute DNA**
   Pipette 200 µL of the Elution Solution directly into the center of the column. Centrifuge for 1 minute at ≥6500 x g to elute the DNA. To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Solution, then centrifuge.

   Optional: A second eluate can be collected by repeating step 9 with an additional 200 µL of Elution Solution and eluting in a new 2 mL collection tube (not provided) or into the same 2 mL collection tube as used for the first eluate. The eluate contains pure genomic DNA. For short term storage of the DNA, 2–8 °C is recommended. For longer-term storage, –20 °C is recommended. Avoid freezing and thawing, which causes breaks in the DNA strand. The Elution Solution will help stabilize the DNA at these temperatures.

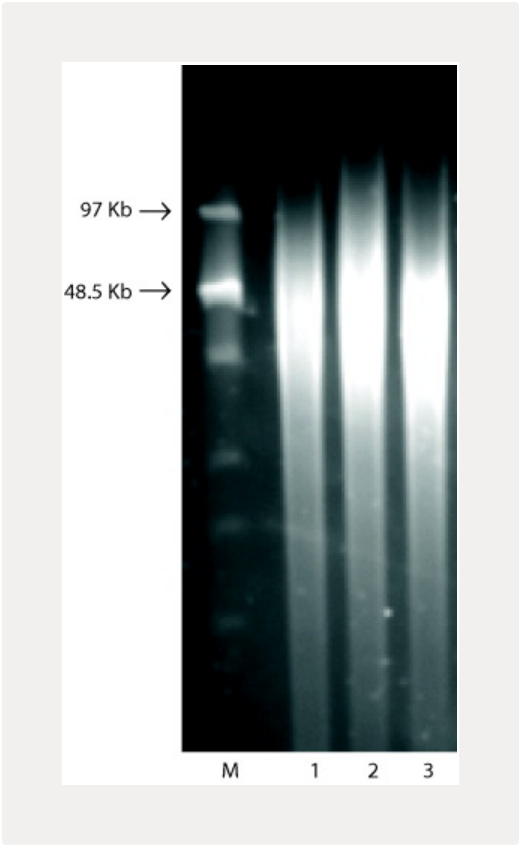


Figure 1. PFGE of Bacterial gDNA isolated with GenElute™ Bacterial gDNA Kit.
Purified genomic DNA was isolated from various bacterial species using the GenElute™ Bacterial Genomic DNA kit. A 1 µg aliquot of DNA from each respective bacterial sample was resolved on a 1% agarose gel in 0.5X TBE at 150 volts for 16 hours using a BioRad CHEF DRII system. The initial pulse time was 2 seconds, the final pulse time was 13 seconds, the start ratio was 1.0, pump speed was set at 70, and PFGE was carried out at 4 °C. M represents the 0.1–200 kb Pulse marker (Cat. No. **D2291**).

Lanes

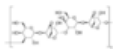
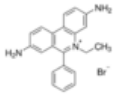
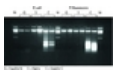
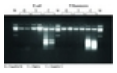
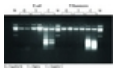
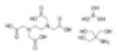
1. E. coli
2. P. fluorescens
3. B. subtilis

Troubleshooting

Problem	Cause	Solution
The lysozyme is difficult to dissolve	The solution is inadequately mixed.	Pipette up and down to dissolve the lysozyme as opposed to vortexing. Excess vortexing will cause foaming and reduce lysozyme solubility. The lysozyme may not dissolve readily. It does not need to be completely dissolved prior to use as it will dissolve during the 37 °C incubation.
The binding column is clogged	The sample is too large.	In the future, use fewer cells (≥ 1 × 1010 cells/mL). To salvage the current preparation, increase the g-force and/or spin longer until the lysate passes through the binding column. The yield of genomic DNA may be reduced.
The lysate appears to be very gelatinous prior to loading onto the column.	The sample is too large.	In the future, use fewer cells (≥ 1 × 1010 cells/mL). Extend the incubation time and/or increase the amount of Proteinase K Solution (step 3a) or Lysozyme Solution (step 3b), depending on whether the gram-negative or gram-positive procedure is performed. For example, double the incubation time as well as the amount of enzyme.
The yield of genomic DNA is low.	The sample is old.	The yield will vary among different species and strains of bacteria. It may be necessary to use bacterial cultures before they reach maximum density or as they become fully confluent.
	The cells are lysed insufficiently.	Extend the incubation time and/or increase the amount of Proteinase K Solution (step A3) or Lysozyme Solution (step B3), depending on whether the gram-negative or gram-positive procedure is performed. For example, double the incubation time as well as the amount of enzyme.
	The lysate/ethanol mixture is not homogenous.	To ensure a homogeneous solution, vortex 5–10 seconds before applying to the binding column. If minimally sheared genomic DNA is desired in downstream applications, e.g., if using the end product for long amplification PCR, mix with gentle pipetting or inversion until homogeneous instead of vortexing.
	The DNA elution is incomplete.	Confirm that the DNA was eluted in 200 µL of Elution Solution. The DNA yield may be improved by incubating the Elution Solution for 5 minutes at room temperature after it is added to the column. A second and third elution using 200 µL of Elution Solution may be performed.
	Ethanol was omitted during binding.	Check that the ethanol was added in step 6 before applying the sample to the binding column in step 7.
	The eluate contains residual ethanol from the wash.	Ethanol from the final wash must be eliminated before eluting the DNA. Spin longer or a second time to dry the membrane. If eluate containing ethanol contacts the binding column, repeat the centrifugation step before eluting the DNA.
	The Wash Solution Concentrate was not diluted before use.	Check that the Wash Solution Concentrate was properly diluted with ethanol before use.
	Water was used for elution instead of the Elution Solution.	The Elution Solution is recommended for optimal yield and storage of the purified DNA. If water is used to elute the DNA, confirm that the pH is at least 7.0, to avoid acidic conditions which may subject the DNA to acid hydrolysis when stored for long periods of time.
Purity of the DNA is lower than expected; A260/A280 ratio is too low.	The sample was diluted in water.	Use either Elution Solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0) or 10 mM Tris-HCl, pH 8.0–8.5 as the eluant.
	The background reading is high due to silica fines.	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purity of the DNA is lower than expected; A260/A280 ratio is too high.	The genomic DNA is contaminated with RNA.	Include an RNase A treatment step in future isolations or treat the final product with RNase A Solution and repurify. It may be necessary to extend the RNase A incubation time in steps A2 and B3 to completely digest the residual RNA.
The DNA is sheared.	The genomic DNA was handled improperly.	This kit was designed to eliminate DNA precipitation and resuspension, common steps found in other genomic DNA kits that can lead to shearing. All pipetting steps should be executed as gently as possible. Wide-orifice pipette tips are recommended to help eliminate shearing. If minimally sheared genomic DNA is desired in downstream applications, e.g., if using the end product for long amplification PCR, mix with gentle pipetting or inversion until homogeneous instead of vortexing.

The cells are old.	Cultures grown for an extended time may lyse prematurely when exposed to cell wall lysing enzymes, resulting in the release of endogenous nucleases and subsequent DNA degradation. Begin with fresh cultures. Downstream applications are inhibited.
Ethanol is carried over the final genomic DNA preparation.	After the final wash of the binding column (step 9), do not allow the eluate to contact the column. Re-spin the column, if necessary, after emptying the collection tube, for an additional 1 minute at maximum speed (12,000–16,000 × g).
Salt is carried over into final genomic DNA preparation.	Make sure that the binding column is transferred to a new 2 mL collection tube before adding the Wash Solutions in steps 8 and 9.

Materials

Product #	Image	Description	Molecular Formula	Add to Cart
A9539		Agarose BioReagent, for molecular biology, low EEO		pricing
E1510		Ethidium bromide solution BioReagent, for molecular biology, 10 mg/mL in H_2O	$C_{21}H_{20}BrN_3$	pricing
E7023	CH_3CH_2OH	Ethyl alcohol, Pure 200 proof, for molecular biology	C_2H_6O	pricing
459836	CH_3CH_2OH	Ethyl alcohol, Pure 200 proof, anhydrous, ≥99.5%	C_2H_6O	pricing
E7148	CH_3CH_2OH	Ethyl alcohol, Pure 190 proof, for molecular biology	C_2H_6O	pricing
NA2100		GenElute™ Bacterial Genomic DNA Kits sufficient for 10 purifications		pricing
NA2110		GenElute™ Bacterial Genomic DNA Kits sufficient for 70 purifications		pricing
NA2120		GenElute™ Bacterial Genomic DNA Kits sufficient for 350 purifications		pricing
L7386		Lysostaphin from *Staphylococcus staphylolyticus* lyophilized powder, Protein 50-70 % by biuret, ≥500 units/mg protein		pricing
L4919		Lysozyme from chicken egg white BioUltra, lyophilized powder, ≥98% (SDS-PAGE), ≥40,000 units/mg protein		pricing
M9901		Mutanolysin from *Streptomyces globisporus* ATCC 21553 lyophilized powder, ≥4000 units/mg protein (biuret), Chromatographically purified		pricing
P2308		Proteinase K from *Tritirachium album* lyophilized powder, BioUltra, ≥30 units/mg protein, for molecular biology		pricing
T6400		Tris-Borate-EDTA buffer 5× Concentrate		pricing
W4502	H_2O	Water Molecular Biology Reagent	H_2O	pricing

References

1. Sambrook, J. F., and Russell, D., *Molecular Cloning: A Laboratory Manual*, 3rd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 2001).
2. Birren, B., and Lai, E., *Pulsed Field Gel Electrophoresis: A Practical Guide* (Academic Press, San Diego, CA, 1993).

Appendix 1

Note: All centrifugation speeds are given in units of g. Please refer to Table 2 for information on converting g-force to RPM. If centrifuges/rotors for the required g-forces are not available, use the maximum g-force possible and increase the spin time

proportionally. Spin until all liquid passes through the column.

Table 2. Conversion of Centrifugal Force (in units of g) to RPM for Common Rotors

Centrifuge	Rotor	Tubes (max)	Radius (cm)	rpm at 6,500 x g	rpm at 12,000 x g	rpm at 16,000 x g
Eppendorf 5410	-	12	5.8	10,012	13,555	15,652
5415C	F45-18-11	18	7.3	8,924	12,124	14,000
5415D&R	F45-24-11	24	8.3	8,369	11,392	13,155
5417C,D,&R	F45-30-11	30	9.5	7,823	10,634	12,279

See table above for spin speeds in RPM for selected common centrifuges and rotors. The correct RPM for unlisted rotorscan be calculated using the formula:

$$rpm = \sqrt{RCF / 1.118 \times 10^{-5} r}$$

where RCF = required gravitational acceleration (relative centrifugal force) in units of g;
r = radius of the rotor in cm;
RPM = the number of revolutions per minute required to achieve the necessary g-force

Appendix 2

Table 3. Typical DNA Yield with the GenElute Bacterial Genomic DNA Kit

Source	Type of Media	Amount of Overnight Culture	OD_{600} per mL Overnight Culture*	Typical DNA Yield (with RNase Treatment)**
Escherichia coli, ATCC# 11775	Terrific broth (**T9179**)	0.8 mL	12.5	20 µg
Escherichia coli, ATCC# 11775	Terrific broth (**L7658**)	1.5 mL	5	20 µg
Escherichia coli DH10B	Terrific broth (**L7658**)	1.0 mL	5	15 µg
Pseudomonas fluorescens, ATCC# 13525	Terrific broth (**T9179**)	0.8 mL	16	25 µg
Pseudomonas fluorescens, ATCC# 13525	Terrific broth (**N7519**)	1.5 mL	2	20 µg
Bacillus subtilis, ATCC# 6051	Terrific broth (**T1438**)	1.5 mL	6	25 µg
Streptococcus mutans, ATCC# 35668	Terrific broth (**T1438**)	1.5 mL	1.3	15 µg***
Streptococcus mutans, ATCC# 14990	Terrific broth (**N7519**)	1.5 mL	2	8 µg****

* Values adjusted for dilution factor. All readings were obtained using a Varian Cary® 100 Spectrophotometer.
** Based on performing two 200 µL elutions.
*** Lysozyme Solution was supplemented with 250 units/mL of mutanolysin (**M9901**).
**** Lysozyme Solution was supplemented with 200 units/mL of lysostaphin (**L7386**).

Precautions and Disclaimer

The GenElute Bacterial Genomic DNA Kit is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.