User Guide

Catalog Nos.
NA2100
NA2110
NA2120

GenElute™ Bacterial Genomic DNA Kit

sigma.com

Ordering Information

Cat. No.	Product Description	Pkg Size
NA2100	GenElute Bacterial Genomic DNA Kit	10 preps
NA2110	GenElute Bacterial Genomic DNA Kit	70 preps
NA2120	GenElute Bacterial Genomic DNA Kit	350 preps

Related Products

Cat. No.	Product Description	Pkg Size
G1N10	GenElute Mammalian Genomic DNA Miniprep	10 preps
G1N70	GenElute Mammalian Genomic DNA Miniprep	70 preps
G1N350	GenElute Mammalian Genomic DNA Miniprep	350 preps
NA1020	GenElute PCR Clean-Up Kit	70 purifications

To reorder product call 1-800-325-3010, visit our Web site at sigma-aldrich.com, or contact your local sales representative.

GenElute Bacterial Genomic DNA Kit

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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	Cat. No.	NA2100 10 Preps	NA2110 70 Preps	NA2120 350 Preps
Gram-Positive Lysis Solution	**L7539**	3 mL	20 mL	90 mL
Lysis Solution T (equivalent to Buffer STL for GenElute Mammalian Genomic DNA Kit)	**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 × 5 mg	3 × 10 mg	2 × 100 mg
RNase A Solution	**R6148**	0.25 mL	1.7 mL	8 mL
GenElute Nucleic Acid Binding Columns	**CP9471***	10 each	70 each	5 × 70 each
Collection Tubes, 2.0 mL capacity	**T5449**	3 × 10 each	3 × 70 each	15 × 70 each

*MilliporeSigma continually seeks ways to improve our products. Please note that the product code for the GenElute Nucleic Acid Binding Columns has changed from C9471 to CP9471. This change has been made to streamline and make more consistent, all the GenElute products across the line. The performance and functionality of C9471 and CP9471 binding columns are equivalent.

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.

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 Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

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**

   For use with both gram-positive and gram-negative bacteria.

2. **Preheat a water bath or heating block to 37 °C**

   For use with gram-positive bacteria only.

3. **Thoroughly Mix Reagents**

   Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and cool to room temperature before use.

4. **Dilute Wash Solution Concentrate**

   Dilute the Wash Solution Concentrate (**B6553**) 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 the ethanol.

5. **Reconstitute Proteinase K**

   Dissolve the powder in one bottle of Proteinase K (**P2308**) 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 Solution and Lysis Solution for storage.

Table 1. Proteinase K Solution Preparation

Cat. No.	Proteinase K	Water
NA2100	5 mg	0.25 mL
NA2110	10 mg	0.5 mL
NA2120	100 mg	5.0 mL

6. Prepare Lysozyme Solution
(for gram-positive bacteria only)

Prepare a 2.115×10^6 unit/mL stock solution of lysozyme (**L4919**) (approximately 45 mg/mL) using the included Gram-Positive Lysis Solution (**L7539**) as the diluent. For example, to make 1 mL of Lysozyme Solution, dissolve 2.115×10^6 units of lysozyme in 1 mL of Gram-Positive Lysis Solution.

Pipette the mixture up and down or vortex to dissolve the lysozyme (see note below). For each DNA preparation to be performed, 200 µL of Lysozyme Solution is required. Make extra solution to account for pipetting error. The Lysozyme Solution should be used on the day of preparation.

Note: Lysozyme may dissolve more readily by pipetting the mixture up and down as opposed to vortexing. Excessive vortexing may cause foaming. The lysozyme may not dissolve readily, in which case it does not need to be completely dissolved prior to use. Genomic DNA yields will not be affected as the lysozyme will dissolve during the 37 °C incubation.

Procedure

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.

See Appendix 1 to convert *g*-force to RPM.

A. Gram-Negative Bacterial Preparation

1a. Harvest Cells

Pellet 1.5 mL of an overnight bacterial broth culture by centrifuging for 2 minutes at 12,000–16,000 × *g*. Remove the culture medium completely and discard.

Note: If bacteria are propagated in rich media such as Terrific broth (**T9179**) it will be necessary to reduce the volume of starting material to 0.5 mL of an overnight bacterial broth culture to avoid overloading the GenElute columns. See Appendix 2 for more information.

2a. Resuspend Cells

Resuspend the pellet thoroughly in 180 µL of Lysis Solution T/ Buffer STL for GenElute Mammalian Genomic DNA Kit (**B6678**). If residual RNA is not a concern, continue with step 3a.

Optional RNase A treatment: If RNA-free genomic DNA is required, add 20 µL of RNase A Solution (**R6148**), mix, and incubate for 2 minutes at room temperature, then continue with step 3a.

3a. Prepare for Cell Lysis

Add 20 µL of the Proteinase K solution to the sample. Mix and incubate for 30 minutes at 55 °C.

4a. Lyse Cells

Add 200 µL of Lysis Solution C (**B8803**), vortex thoroughly (about 15 seconds), and incubate at 55 °C for 10 minutes. A homogeneous mixture is essential for efficient lysis. Continue with step 5.

B. Gram-Positive Bacterial Preparation

1b. Prepare Lysozyme Solution Using Lysozyme from Chicken Egg White (L4919)

Prepare a 2.115×10^6 unit/mL stock solution of lysozyme as described under Preparation Instructions. For each DNA preparation to be performed, 200 µL of Lysozyme Solution is required. Prepare extra solution to account for pipetting error.

Note: If working with *Staphylococcus* species, supplement the Lysozyme Solution with 200 units/mL of lysostaphin (**L7386**). For *Streptococcus* species, supplement the Lysozyme Solution with 250 units/mL of mutanolysin (**M9901**).

2b. Harvest Cells

Pellet 1.5 mL of an overnight bacterial broth culture by centrifuging for 2 minutes at 12,000–16,000 × *g*. Remove the culture medium completely and discard.

Note: If bacteria are propagated in rich media such as Terrific broth (**T9179**), it will be necessary to reduce the volume of starting material to 0.5 mL of overnight bacterial broth culture to avoid overloading the GenElute columns. See Appendix 2 for more information.

3b. Resuspend Cells

Resuspend the pellet thoroughly in 200 µL of Lysozyme Solution (prepared in step 1b) and incubate for 30 minutes at 37 °C.

Optional RNase A treatment: If residual RNA is not a concern, continue with step 4b. If RNA-free genomic DNA is required, add 20 µL of RNase A Solution (**R6148**) and incubate for 2 minutes at room temperature, then continue with step 4b.

4b. Lyse Cells

Add 20 µL of the Proteinase K solution to the sample, followed by 200 µL of Lysis Solution C (**B8803**). Vortex thoroughly (about 15 seconds) and incubate at 55 °C for 10 minutes. A homogeneous mixture is essential for efficient lysis. Continue with step 5.

DNA Isolation from Gram-Positive and Gram-Negative Bacteria

This is a continuation of the procedure from the lysates prepared in steps 1–4a and/or steps 1–4b.

5. Column Preparation

Assemble a binding column (**CP9471**) with a 2 mL collection tube (**T5449**). Add 500 uL of Column Preparation Solution (**C2112**) to the binding column and centrifuge at 12,000 X *g* for 1 minute. Discard the flow-through liquid, but retain the collection tube.

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

6. Prepare for Binding

Add 200 µL of ethanol (95–100%) to the lysate from step 4a or 4b and mix thoroughly by vortexing for 5–10 seconds. A homogeneous mixture is essential.

7. Load Lysate

Transfer the entire contents of the tube into the treated binding column from step 5. Use a wide bore pipette tip to reduce shearing the DNA when transferring contents into the binding column. Centrifuge at > 6500 × *g* for 1 minute. Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 mL collection tube.

8. First Wash

Add 500 µL of Wash Solution 1 (**W0263**) to the column and centrifuge for 1 minute at ≥ 6500 × *g*. Discard the flow-through liquid, but retain the collection tube.

9. Second Wash

Important Reminder: *Verify that ethanol has been added to the bottle of Wash Solution Concentrate.*

Add 500 µL of Wash Solution Concentrate (**B6553**), *previously diluted with ethanol*, to the column and centrifuge for 3 minutes at maximum speed (12,000–16,000 × *g*) to dry the column. The column must be free of ethanol before eluting the DNA. Centrifuge the column for an additional 1 minute at maximum speed if residual ethanol is seen. You may empty and re-use the collection tube if you need this additional centrifugation step. Finally, discard the collection tube containing the flow-through liquid and place the binding column in a new 2 mL collection tube.

10. Elute DNA

Pipette 200 µL of the Elution Solution (**B6803**) directly onto the center of the column; centrifuge for 1 minute at ≥ 6500 × *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 elution can be collected by repeating step 10 with an additional 200 µL of Elution Solution and eluting into a new 2 mL collection tube (not provided) or into the same 2 mL collection tube as used for the first eluate. The yield can be improved by up to 30% when performing a second elution

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.

DNA Precipitation (Optional)

The GenElute Bacterial Genomic DNA Kit is designed so the DNA always remains in solution, avoiding resuspension issues. However, if you find it necessary to concentrate the DNA, ethanol precipitation in the presence of sodium acetate is recommended.[1]

Results

The concentration and quality of the genomic DNA can be determined by spectrophotometric analysis and agarose gel electrophoresis. Dilute the DNA in TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0–8.5) and measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. The absorbance at 260 nm and 280 nm should be between 0.1 and 1.0 (or within the linear range of your spectrophotometer). The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/mL of double-stranded DNA. The $A_{260}–A_{320}/A_{280}–A_{320}$ ratio should be 1.6–1.9.

The size and quality of the DNA can be determined by agarose gel electrophoresis.[1] A gel containing 0.8% agarose (**A9539**) in 0.5X TBE Buffer (**T6400**) works well for the resolution of genomic DNA. The DNA can be visualized by staining with an intercalating dye such as ethidium bromide (**E1510**) and measured against a known DNA marker such as Lambda DNA *Hin*d III digest (**D9780**). The genomic DNA should migrate as a single, high molecular weight band with very little evidence of shearing. A more precise determination of the size of the DNA can be made by pulsed-field gel electrophoresis.[2]

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).

Troubleshooting Guide

The lysozyme is difficult to dissolve.	**Cause** — The solution is inadequately mixed. **Solution** — 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.	**Cause** — The sample is too large. **Solution** — In the future, use fewer cells ($\geq 1 \times 10^{10}$ 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.	**Cause** — The sample is too large. **Solution** — In the future, use fewer cells ($\geq 1 \times 10^{10}$ 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.	**Cause** — The sample is old. **Solution** — 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.
	Cause — The cells are lysed insufficiently. **Solution** — 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.
	Cause — The lysate/ethanol mixture is not homogenous. **Solution** — 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.
	Cause — The DNA elution is incomplete. **Solution** — 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.

The yield of genomic DNA is low.	**Cause** — Ethanol was omitted during binding. **Solution** — Check that the ethanol was added in step 6 before applying the sample to the binding column in step 7.
	Cause — The eluate contains residual ethanol from the wash. **Solution** — 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.
	Cause — The Wash Solution Concentrate was not diluted before use. **Solution** — Check that the Wash Solution Concentrate was properly diluted with ethanol before use.
	Cause — Water was used for elution instead of the Elution Solution. **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; A_{260}/A_{280} ratio is too low.	**Cause** — The sample was diluted in water. **Solution** — 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.
	Cause — The background reading is high due to silica fines. **Solution** — 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; A_{260}/A_{280} ratio is too high.	**Cause** — The genomic DNA is contaminated with RNA. **Solution** — 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 2a and 3b to completely digest the residual RNA.
The DNA is sheared.	**Cause** — The genomic DNA was handled improperly. **Solution** — 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.

	Cause — The cells are old. **Solution** — 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.	**Cause** — Ethanol is carried over the final genomic DNA preparation. **Solution** — 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*).
	Cause — Salt is carried over into final genomic DNA preparation. **Solution** — 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.

Related Products	Catalog No.	Related Products	Catalog No.
Agarose	**A9539**	Deoxynucleotide (dNTP) Mix, 10 mM	**D7295**
Ethanol, 95+%	**E7148**	Lambda DNA *Eco*R I *Hin*d III digest	**D9281**
Ethanol, 100%	**E7023**	Gel Loading Solution	**G2526**
Tris-EDTA Buffer, 100× Concentrate	**T9285**	*Eco*R I (10,000 units/mL)	**R6265**
Ethidium Bromide, 10 mg/mL	**E1510**	Lysozyme	**L4919**
Microcentrifuge Tubes, 1.5 mL	**T9661**	Mutanolysin	**M9901**
AccuTaq™ LA DNA Polymerase	**D8045**	Lysostaphin	**L7386**
Taq DNA Polymerase	**D1806** **D4545**	TBE Buffer, 5× Concentrate	**T6400**
Water, Molecular Biology Reagent	**W4502**		

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 × *g*	RPM at 12,000 × *g*	RPM at 16,000 × *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**).

Experienced User Protocol — Gram-Negative Bacterial Preparation

1 Harvest Cells

- ❑ Pellet 1.5 mL of bacterial broth culture at 12,000–16,000 × *g* for 2 minutes, discard media. When using enriched media, please see Procedure, step 1a, on page 5.

2 Resuspend Cells

- ❑ Resuspend pellet in 180 µL Lysis Solution T.
  Optional: Add 20 µL RNase A, incubate RT for 2 minutes.

3 Lyse Cells

- ❑ Add 20 µL Proteinase K to cell suspension, vortex or pipet to mix. Incubate at 55 °C for 30 min.
- ❑ Add 200 µL Lysis Solution C, vortex or pipette to mix. Incubate at 55 °C for 10 minutes.

4 Prepare Column

- ❑ Add 500 µL of Column Preparation Solution to each binding column.
- ❑ *Spin at ≥12,000 × g for 1 minute.* Discard flow-through.

5 Bind DNA to Column

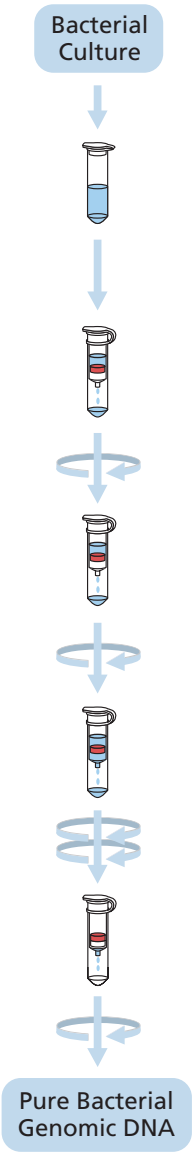
- ❑ Add 200 µL ethanol to the lysed cells, vortex or invert to mix.
- ❑ Transfer EtOH mixture to binding column. *Spin at ≥ 6500 × g for 1 minute.*

6 Wash Column

- ❑ Transfer column to new collection tube. Add 500 µL Wash Solution 1 to column. Spin at ≥ 6500 × *g* for 1 minute. Discard flow-through.
- ❑ Add 500 µL Wash Solution Concentrate to column. *Spin at ≥12,000 × g for 3 minutes to dry column.*

7 Elute DNA

- ❑ Transfer column to new collection tube. Add 200 µL of Elution Solution. *Spin at ≥ 6500 × g for 1 minute.*
  Optional: Repeat in new (not provided) or same tube.



Experienced User Protocol — Gram-Positive Bacterial Preparation

❑ **Prepare Lysozyme Solution**
(Lysozyme sold separately — Catalog No. L4919)
Prepare a 2.115×10^6 unit/mL Lysozyme Solution using the included Gram-Positive Lysis Buffer as the diluent. 200 µL of Lysozyme Solution is needed for each prep. Make extra to account for pipetting error.

1 Harvest Cells

❑ Pellet 1.5 mL of bacterial broth culture at 12,000–16,000 × *g* for 2 minutes, discard media. When using enriched media, please see Procedure, step 2b, on page 6.

2 Digest Cell Wall

❑ Resuspend pellet in 200 µL Lysozyme Solution and incubate at 37 °C for 30 minutes.
Optional: Add 20 µL RNase A, incubate RT for 2 min.

3 Lyse Cells

❑ Add 20 µL Proteinase K and 200 µL Lysis Solution C to cell suspension, vortex or pipette to mix. Incubate at 55 °C for 10 minutes.

4 Prepare Column

❑ Add 500 µL of Column Preparation Solution to each binding column.
❑ *Spin at ≥12,000 × g for 1 minute.* Discard flow-through.

5 Bind DNA to Column

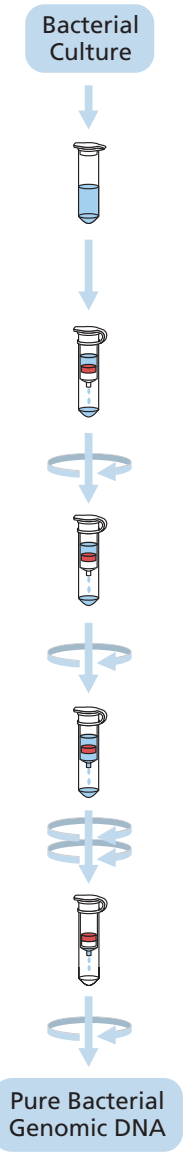
❑ Add 200 µL ethanol to the lysed cells, vortex or invert to mix.
❑ Transfer EtOH mixture to binding column. *Spin at ≥ 6500 × g for 1 minute.*

6 Wash Column

❑ Transfer column to new collection tube. Add 500 µL Wash Solution 1 to column. Spin at ≥ 6500 × *g* for 1 minute. Discard flow-through.
❑ Add 500 µL Wash Solution Concentrate to column. *Spin at ≥12,000 × g for 3 minutes to dry column.*

7 Elute DNA

❑ Transfer column to new collection tube. Add 200 µL of Elution Solution. *Spin at ≥ 6500 × g for 1 minute.*
Optional: Repeat in new (not provided) or same tube.



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