User Guide

Catalog Nos.
NA2010
NA2020

GenElute™ Blood Genomic DNA Kit

sigma.com

Ordering Information

Cat. No.	Product Description	Pkg Size
NA2010	GenElute Blood Genomic DNA Kit	70 preps
NA2020	GenElute Blood Genomic DNA Kit	350 preps

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

GenElute Blood Genomic DNA Kit

Table of Contents

Product Description

Sigma's GenElute™ Blood Genomic DNA Kit provides a simple and convenient way to isolate pure genomic DNA from fresh or aged (older than 24 hours) whole blood. The kit combines the advantages of silica binding with a microspin format, and eliminates the need for expensive resins, alcohol precipitation, and hazardous organic compounds such as phenol and chloroform. The starting material is lysed in a chaotropic salt-containing solution to insure the thorough denaturation of macromolecules. The addition of ethanol causes the DNA to bind when the lysate is spun through a silica membrane in a microcentrifuge tube. A Prewash Solution is provided to help remove contaminants that are associated with aged (older than 24 hours) whole blood samples. After washing to remove contaminants, the DNA is eluted in 200 µL of a Tris-EDTA solution.

The expected yields of genomic DNA will vary depending on the amount and nature of the starting material used (for example, 4–10 µg of RNase A-treated DNA can be isolated from 200 µL of fresh whole blood in less than one hour). DNA purified with this 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, Southern blots, and sequencing reactions.

Reagents Provided	Cat. No.	NA2010 70 Preps	NA2020 350 Preps
Resuspension Solution	**P3980**	20 mL	100 mL
Lysis Solution C	**B8803**	20 mL	90 mL
Column Preparation Solution	**C2112**	60 mL	225 mL
Prewash Solution Concentrate	**P6365**	22.5 mL	90 mL
Wash Solution Concentrate	**B6553**	20 mL	90 mL
Elution Solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0)	**B6803**	35 mL	180 mL
Proteinase K	**P2308**	3 × 10 mg	2 × 100 mg
RNase A Solution	**R6148**	1.7 mL	8 mL
GenElute Miniprep Binding Columns	**CP9471***	70 each	5 × 70 each
Collection Tubes, 2.0 mL capacity	**T5449**	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

- 55 °C water bath or heating block
- Pipette tips (aerosol barrier recommended)
- 1.5 mL microcentrifuge tubes for lysis
- Microcentrifuge (2 mL tube, rotor equiped)**
- Ethanol (95–100%), Catalog Nos. **E7148**, **E7023**, or **459836**
- Molecular Biology Reagent Water, Catalog No. **W4502**

****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 Blood Genomic DNA Kit is for laboratory use only, not for drug, household, or other uses. The Lysis Solution C contains a chaotropic salt, which is an irritant. The Column Preparation Solution is an irritant. Avoid contact with skin. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagent provided with the kit. 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**

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 Concentrate**

   Dilute the Prewash Solution Concentrate (**P6365**) 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 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 ethanol.

5. **Dissolve the 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 and Lysis Solutions for storage.

Table 1. Proteinase K Solution Preparation

Cat. No.	Proteinase K	Water
NA2010	10 mg	0.5 mL
NA2020	100 mg	5.0 mL

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.

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

Centrifuge	Rotor	Tubes (max)	Radius (cm)	rpm at 300 × *g*	rpm at 6,500 × *g*	rpm at 12,000 × *g*	rpm at 16,000 × *g*
Eppendorf							
5410	–	12	5.8	2,143	10,012	13,555	15,652
5415C	F45-18-11	18	7.3	1,917	8,924	12,124	14,000
5415D&R	F45-24-11	24	8.3	1,801	8,369	11,392	13,155
5417C,D,&R	F45-30-11	30	9.5	1,681	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

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 Resuspension Solution (**R3980**) 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 (**R6148**) and incubate for 2 minutes at room temperature; continue with step 3.

3. Lyse Cells

Add 200 µL of Lysis Solution C (**B8803**) 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

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 × *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.

5. Prepare for Binding

Add 200 µL of ethanol (95–100%) to the lysate from step 3 and 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 binding column from step 4. Use a wide bore pipette tip to reduce shearing of 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.

7. First Wash

Prior to first use, dilute both the Prewash Solution Concentrate (**P6365**) and the Wash Solution Concentrate (**B6553**) with ethanol as described in the Preparation Instructions. Add 500 µL of either the diluted Prewash Solution or the diluted Wash Solution to the column and centrifuge for 1 minute at ≥6500 × *g*. Discard the flow-through liquid, but retain the collection tube.

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

8. Second Wash

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.

9. 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 9 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 Blood Genomic DNA Kit is designed so that the DNA always remains in solution, which avoids resuspension issues. However, if it is 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 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 doublestranded 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 0.5× 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.; et al. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press, Plainview, NY, 1989.
2. Birren, B.; Lai, E. *Pulsed Field Gel Electrophoresis: A Practical Guide;* Academic Press: San Diego, CA, 1993.

Troubleshooting Guide

Binding column is clogged.	**Cause** — Sample is too large. **Solution** — In the future, use a smaller quantity of whole blood. 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.
Poor or low genomic DNA recovery.	**Cause** — Sample is old or degraded. **Solution** — The yield will vary among individual samples of fresh or aged (older than 24 hours) whole blood. Use whole blood within a few hours of collection for best results. If samples are being stored for future use, whole blood may be kept at 4 °C for at least 3 months.
	Cause — Lysate/ethanol mixture is not homogeneous. **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.

Poor or low genomic DNA recovery.	**Cause** — DNA elution is incomplete. **Solution** — Confirm that the DNA was eluted in 200 µL of Elution Solution. The DNA yield for most types of material 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 also be performed.
	Cause — Ethanol was omitted during binding. **Solution** — Check that the ethanol was added in step 5 before applying the sample to the binding column in step 6.
	Cause — The eluate contains residual ethanol from 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 flow-through liquid containing ethanol contacts the binding column, repeat the centrifugation step before eluting DNA.
	Cause — Prewash and/or Wash Solution Concentrates were not diluted before use. **Solution** — Check that Prewash and Wash Solution Concentrates were properly diluted with ethanol before use.
	Cause — Water was used for elution instead of Elution Solution. **Solution** — Elution Solution is recommended for optimal yields 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** — Eluate was diluted in water for absorbance measurement. **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 diluent.
	Cause — Blood sample was older than 24 hours. **Solution** — In future preparations use the Prewash Solution in the first wash step.
	Cause — Background reading is high due to silica fines. **Solution** — Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat absorbance readings.
	Cause — Purification is incomplete due to column overloading or inadequate lysis. **Solution** — Reduce the initial volume of blood or increase the lysis time (step 3) while monitoring the lysis visually.
Purity of the DNA is lower than expected (A_{260}/A_{280} ratio is too high).	**Cause** — Genomic DNA is contaminated with RNA. **Solution** — Include an RNase A treatment step in future isolations or treat final product with RNase A Solution and repurify.

DNA is sheared.	**Cause** — Genomic DNA was handled improperly. **Solution** — This kit is 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 — Blood sample is old, degraded, or has undergone repeated freeze/thaw cycles. **Solution** — Old starting material may yield degraded DNA in the eluate. For best results, fresh whole blood preparations should be used immediately. Alternatively, whole blood can be stored at 4 °C for up to 3 months.
Downstream applications are inhibited.	**Cause** — Ethanol is carried over into the final genomic DNA preparation. **Solution** — After the final wash of the binding column (step 8), do not allow the flow-through liquid to contact the column. Re-spin the column for 1 additional minute at maximum speed (12,000–16,000 × *g*), if necessary, after emptying the collection tube.
	Cause — Salt is carried over into the final genomic DNA preparation. **Solution** — Make sure that the binding column is transferred to a new 2 mL collection tube before adding the Prewash or Wash Solutions in step 7.

Related Products	Cat. No.	Related Products	Cat. No.
Agarose	**A9539**	AccuTaq™ LA DNA Polymerase	**D8045**
Ethidium bromide, 10 mg/mL	**E1510**	JumpStart™ REDTaq® ReadyMix™ PCR reaction mix	**P0982**
Taq DNA Polymerase	**D1806, D4545**	REDTaq® Genomic DNA Polymerase	**D8312**
Deoxynucleotide (dNTP) Mix, 10 mM	**D7295**	REDAccuTaq® LA DNA Polymerase	**D4812**
Lambda DNA *Hin*d III digest	**D9780**	*Eco*R I (10,000 units/mL)	**R6265**
Gel Loading Solution	**G2526**	TBE Buffer, 5× Concentrate	**T6400**

Appendix: Larger Volumes of Blood

Up to 500 µL of blood can be used with this kit. Reagents must be increased accordingly. (Lysis Solution C and ethanol additions should be approximately 50 µL over the volume of whole blood added in the preparation.) This will decrease the number of preparations you can get from the kit. The binding column will have to be filled and spun several times to load all of the lysate from step 5, depending upon the volume used. For example, add 50 µL of Proteinase K to a 2 mL tube, 500 µL of whole blood, 40 µL of RNase A, and 550 µL of Lysis Solution C. Mix and incubate at 55 °C for 10 minutes. Add 550 µL of 95–100% ethanol and mix. Load onto the binding column (3 × 600 µL) and spin as in step 6. Continue with steps 7–9 as noted in the Procedure.

Experienced User Protocol

1 Release DNA

- ❒ Enzynmatic digestion.

2 Prepare Column

- ❒ Add solution and spin.

3 Bind DNA

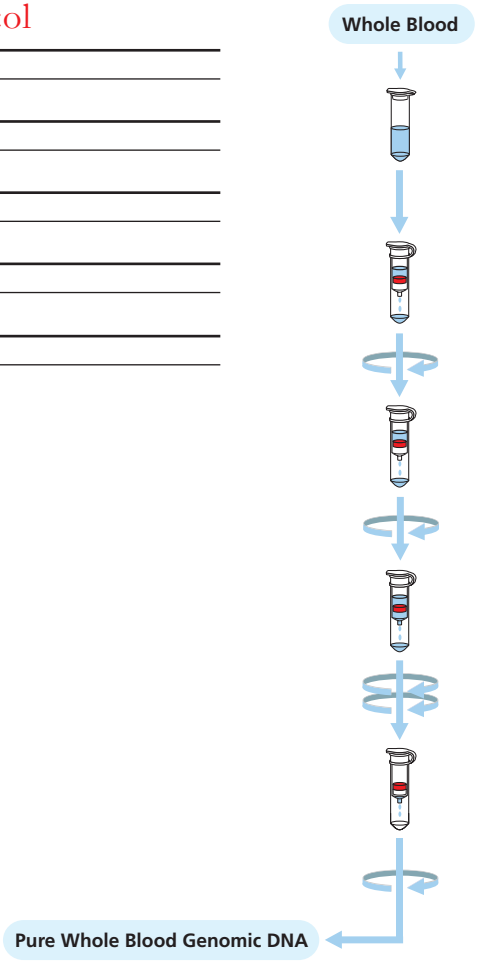
- ❒ Add ethanol and spin.

4 Wash Column

- ❒ Wash and spin twice.

5 Elute DNA

- ❒ Spin 1 minute.



Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service (800) 325-5832 • sigma-aldrich.com/techservice
Development/Custom Manufacturing Inquiries SAFC® (800) 244-1173
Safety-related Information sigma-aldrich.com/safetycenter

3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

SIGMA-ALDRICH®

2017-06567
1097