

GenElute™ Blood Genomic DNA Kit
Product Information

 Product Codes **NA 2000, NA 2010, and NA 2020**
TECHNICAL BULLETIN
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	Product Code	NA 2000 10 Preps	NA 2010 70 Preps	NA 2020 350 Preps
Resuspension Solution	P 3980	3 ml	20 ml	100 ml
Lysis Solution C	B 8803	2.5 ml	20 ml	90 ml
Column Preparation Solution	C 2112	7 ml	60 ml	225 ml
Prewash Solution Concentrate	P 6365	4.5 ml	22.5 ml	90 ml
Wash Solution Concentrate	B 6553	2.5 ml	20 ml	90 ml
Elution Solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0)	B 6803	5 ml	35 ml	180 ml
Proteinase K	P 2308	1 x 5 mg	3 x 10 mg	2 x 100 mg
RNase A Solution	R 6148	0.25 ml	1.7 ml	8 ml
GenElute Miniprep Binding Columns in tubes	C 9471	10 each	70 each	5 x 70 each
Collection Tubes, 2.0 ml capacity	T 7813	3 x 10 each	3 x 70 each	15 x 70 each

Reagents and Equipment Required But Not Provided

- 55 °C water bath or heating block
- Pipette tips with aerosol barrier (recommended)
- 1.5 ml microcentrifuge tubes for lysis, Product Code T 9661
- Microcentrifuge (with rotor for 2 ml tubes)
- Ethanol (95-100%), Product Codes E 7148, E 7023, or 45,983-6
- Molecular Biology Reagent Water, Product Code W 4502

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

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

Before beginning the procedure, do the following:

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 concentrate with 5.5 ml (10 prep package), 27.5 ml (70 prep package), 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 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.

Table 1.
Proteinase K Solution Preparation

Kit Code	Proteinase K	Water
NA 2000	5 mg	0.25 ml
NA 2010	10 mg	0.5 ml
NA 2020	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 x g	RPM at 6,500 x g	RPM at 12,000 x g	RPM at 16,000 x 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 2 for spin speeds in rpm for selected common centrifuges and rotors. The correct rpm for unlisted rotors can 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 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 $\geq 6,500$ 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 $\geq 6,500$ 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 $\geq 6,500$ 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.

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 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 (Product Code A 9539) in 0.5x TBE Buffer (Product Code T 6400) works well for the resolution of genomic DNA. The DNA can be visualized by staining with an intercalating dye such as ethidium bromide (Product Code E 1510) and measured against a known DNA marker such as Lambda DNA *Hind* III digest (Product Code D 9780). 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

Troubleshooting Guide

Problem	Cause	Solution
Binding column is clogged.	Sample is too large.	In the future, use a smaller quantity of whole blood. To salvage the current preparation, increase the <i>g</i> -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	Sample is old or degraded.	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.
	Lysate/ethanol mixture is not homogeneous.	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.
	DNA elution is incomplete.	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.
	Ethanol was omitted during binding.	Check that the ethanol was added in step 5 before applying the sample to the binding column in step 6.
	The eluate contains residual ethanol from wash.	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.
	Prewash and/or Wash Solution Concentrates were not diluted before use.	Check that Prewash and Wash Solution Concentrates were properly diluted with ethanol before use.

Troubleshooting Guide (continued)

Problem	Cause	Solution
Poor or low genomic DNA recovery (continued)	Water was used for elution instead of Elution 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).	Eluate was diluted in water for absorbance measurement.	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.
	Blood sample was older than 24 hours.	In future preparations use the Prewash Solution in the first wash step.
	Background reading is high due to silica fines.	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat absorbance readings.
	Purification is incomplete due to column overloading or inadequate lysis.	Reduce the initial volume of blood or increase the lysis time (step 3) while monitoring the lysis visually.
Purity of DNA is lower than expected (A_{260}/A_{280} ratio is too high).	Genomic DNA is contaminated with RNA.	Include an RNase A treatment step in future isolations or treat final product with RNase A Solution and repurify.
DNA is sheared.	Genomic DNA was handled improperly.	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.
	Blood sample is old, degraded, or has undergone repeated freeze/thaw cycles.	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.	Ethanol is carried over into the final genomic DNA preparation.	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 x g), if necessary, after emptying the collection tube.
	Salt is carried over into the final genomic DNA preparation.	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.

Appendix: Larger Volumes of Blood

Up to 500 μ l of blood can be used with this kit. Reagents must be increased accordingly. (Lysis Solution C and ethanol additions should be approximately 50 μ l 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 μ l of Proteinase K to a 2 ml tube, 500 μ l of whole blood, 40 μ l of RNase A, and 550 μ l of Lysis Solution C. Mix and incubate at 55 °C for 10 minutes. Add 550 μ l of 95-100% ethanol and mix. Load onto the binding column (3 x 600 μ l) and spin as in step 6. Continue with steps 7-9 as noted in the Procedure.

References

1. Sambrook, J., *et al.* *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989).
2. Birren, B. and Lai, E. *Pulsed Field Gel Electrophoresis: A Practical Guide* (Academic Press, San Diego, CA, 1993).

Related Products	Product Code	Related Products	Product Code
Agarose	A 9539	AccuTaq™ LA DNA Polymerase	D 8045
Tris-EDTA Buffer, 100x Concentrate	T 9285	KlenTaq LA DNA Polymerase Mix	D 5062
Ethidium Bromide, 10 mg/ml	E 1510	JumpStart™ REDTaq™ ReadyMix™ PCR reaction mix	P 0982
Taq DNA Polymerase	D 1806 D 4545	REDTaq™ Genomic DNA Polymerase	D 8312
Deoxynucleotide (dNTP) Mix, 10 mM	D 7295	REDAccuTaq™ LA DNA Polymerase	D 4812
Lambda DNA <i>Hind</i> III digest	D 9780	<i>Eco</i> R I (40,000 units/ml)	R 4640
Gel Loading Solution	G 2526	TBE Buffer, 5x Concentrate	T 6400

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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