

# User Guide

Catalog Nos.

G1N10

G1N70

G1N350

# GenElute™ Mammalian Genomic DNA Miniprep Kit

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## Ordering Information

Catalog No.	Product Description	Pkg Size
G1N10	GenElute Mammalian Genomic DNA Miniprep Kit	10 preps
G1N70	GenElute Mammalian Genomic DNA Miniprep Kit	70 preps
G1N350	GenElute Mammalian Genomic DNA Miniprep Kit	350 preps

## Related Products

Catalog No.	Product Description	Pkg Size
NA2010	GenElute Blood Genomic DNA Kit	70 preps
NA2020	GenElute Blood Genomic DNA Kit	350 preps
NA2100	GenElute Bacterial Genomic DNA Kit	10 preps
NA2110	GenElute Bacterial Genomic DNA Kit	70 preps
NA2120	GenElute Bacterial Genomic DNA Kit	350 preps

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# GenElute™ Mammalian Genomic DNA Miniprep Kit

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## Product Description

Sigma's GenElute Mammalian Genomic DNA Miniprep Kit provides a simple and convenient way to isolate pure genomic DNA from a variety of cultured cells, tissues (including rodent tails), and fresh whole blood or white blood cells. 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 materials are lysed in a chaotropic salt-containing solution to insure the thorough denaturation of macromolecules. Addition of ethanol causes DNA to bind when the lysate is spun through a silica membrane in a microcentrifuge tube. After washing to remove contaminants, DNA is eluted in 200  $\mu$ L of a Tris-EDTA solution.

Expected yields of genomic DNA will vary depending on the amount and type of starting material used (for example, 15–25  $\mu$ g of RNase A-treated DNA can be isolated from  $2 \times 10^6$  HeLa cells in less than one hour). DNA purified with the 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.

Components	Catalog No.	G1N10 10 Preps	G1N70 70 Preps	G1N350 350 Preps
Resuspension Solution	<b>P3980</b>	3 mL	20 mL	100 mL
Lysis Solution T	<b>B6678</b>	2.5 mL	20 mL	90 mL
Lysis Solution C	<b>B8803</b>	2.5 mL	20 mL	90 mL
Column Preparation Solution	<b>C2112</b>	7 mL	60 mL	225 mL
Wash Solution Concentrate	<b>B6553</b>	2.5 mL	20 mL	90 mL
Elution Solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0)	<b>B6803</b>	5 mL	35 mL	180 mL
Proteinase K	<b>P2308</b>	1 $\times$ 5 mg	3 $\times$ 10 mg	2 $\times$ 100 mg
RNase A Solution	<b>R6148</b>	0.25 mL	1.7 mL	8 mL
GenElute Miniprep Binding Columns in tubes	<b>C9471</b>	10 each	70 each	5 $\times$ 70 each
Collection Tubes, 2.0 mL capacity	<b>T5449 or T7813</b>	4 $\times$ 10 each	4 $\times$ 70 each	20 $\times$ 70 each

## Reagents and Equipment Required But Not Provided

- 55 °C water bath or shaking water bath
- 70 °C water bath or heating block
- Pipette tips with aerosol barrier (recommended)
- 1.5 mL microcentrifuge tube for lysis
- Microcentrifuge (2 mL tube, rotor-equipped)\*
- 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 Mammalian 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

Before beginning the procedure, do the following:

- 1. Preheat a water bath or shaking water bath to 55 °C** For use with tissues, rodent tails, fresh whole blood and white blood cells.
- 2. Preheat a water bath or heating block to 70 °C** For use with cultured cells and tissues.
- 3. Thoroughly mix reagents** Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves and allow to cool to room temperature before use.
- 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 Proteinase K in water** According to Table 1 below, dissolve the powder in one bottle of Proteinase K in water (Catalog No. **W4502**) to obtain a 20 mg/mL stock solution. This 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.  
  
The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis Solutions for storage.

**Table 1. Proteinase K Solution Preparation**

Catalog No.	Proteinase K (mg)	Water (mL)
G1N10	5	0.25
G1N70	10	0.5
G1N350	100	5.05

## Procedure

**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.

### A. Cultured Cell Preparation

#### 1a. Harvest cells

- Attached cell cultures: Release cells with trypsin. Pellet up to  $5 \times 10^6$  cells for 5 minutes at  $300 \times g$ ; remove the culture medium completely and discard. Continue to step 2a.
- Suspension cell cultures: Pellet up to  $5 \times 10^6$  cells for 5 minutes at  $300 \times g$ ; remove the culture medium completely and discard. Continue with step 2a.

**Note:** Cells can be harvested, aliquotted into 1.5 mL microcentrifuge tubes and flash-frozen in liquid nitrogen, then stored at  $-70^\circ\text{C}$  for several months before preparing DNA.

#### 2a. Resuspend cells

Resuspend the pellet thoroughly in 200  $\mu\text{L}$  of Resuspension Solution. If previously frozen, allow the cell pellet to thaw slightly before resuspending. If residual RNA is not a concern, continue with step 3a.

**Optional RNase A treatment:** If RNA-free genomic DNA is required, add 20  $\mu\text{L}$  of RNase A Solution and incubate for 2 minutes at room temperature, then continue with step 3a.

#### 3a. Lyse cells

Add 20  $\mu\text{L}$  of the Proteinase K solution to the sample, followed by 200  $\mu\text{L}$  of Lysis Solution C (**B8803**). Vortex thoroughly (about 15 seconds), and incubate at  $70^\circ\text{C}$  for 10 minutes. A homogeneous mixture is essential for efficient lysis. Continue with step 4.

### B. Mammalian Tissue Preparation

#### 1b. Prepare tissue

Quickly mince and weigh a piece of fresh or frozen tissue. Allow frozen tissue to thaw slightly before slicing, but keep on ice to protect against degradation. Cutting tissue into small pieces enables more efficient lysis. Up to 25 mg of tissue (or 10 mg of spleen, due to the high number of cells per given mass) may be used per preparation. Transfer to a 1.5 mL microcentrifuge tube and continue to step 2b.

**Note:** Tissue can be harvested, aliquotted into 1.5 mL microcentrifuge tubes and flash-frozen in liquid nitrogen, then stored at  $-70^\circ\text{C}$  for several months before preparing the DNA.

### 2b. Digest tissue

Add 180  $\mu\text{L}$  of Lysis Solution T (**B6678**), followed by 20  $\mu\text{L}$  of the Proteinase K solution to the tissue. Mix by vortexing. Incubate the sample at 55 °C until the tissue is completely digested and no particles remain. Vortex occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. After digestion is complete, vortex briefly.

**Optional RNase A treatment:** If residual RNA is not a concern, continue with step 3b. If RNA-free genomic DNA is required, add 20  $\mu\text{L}$  of RNase A Solution and incubate for 2 minutes at room temperature, then continue with step 3b.

### 3b. Lyse cells

Add 200  $\mu\text{L}$  of Lysis Solution C (**B8803**) to the sample; vortex thoroughly (15 seconds). A homogeneous mixture is essential for efficient lysis. Incubate at 70 °C for 10 minutes. Continue with step 4.

## C. Rodent Tail Preparation

### 1c. Prepare rodent tails

Quickly measure and cut a piece of fresh or frozen rodent tail. Allow frozen rodent tail to thaw slightly before cutting, but keep on ice to protect against degradation. Do not use more than 0.6 cm (rat) or 1.2 cm (mouse) tail per preparation. Cut two (mouse) or one (rat) 0.5–0.6 cm lengths of tail and place them in a 1.5 mL microcentrifuge tube; continue to step 2c.

**Note:** Rodent tails can be stored at –20 °C for several months before preparing DNA.

### 2c. Digest tissue

Add 180  $\mu\text{L}$  of Lysis Solution T (**B6678**), followed by 20  $\mu\text{L}$  of the Proteinase K solution to the rodent tail. Mix by vortexing; ensure that the tail is fully submerged. Incubate the sample at 55 °C until the tail tissue is completely digested. Some particles (bone and hair) may remain. Vortex occasionally or use a shaking water bath during incubation for more rapid digestion. Digestion is usually complete in 3 to 6 hours. After digestion is complete, vortex briefly. If residual RNA is not a concern, continue with step 3.

**Optional RNase A treatment:** If RNA-free genomic DNA is required, add 20  $\mu\text{L}$  of RNase A Solution and incubate for 2 minutes at room temperature; continue with step 3c.

### 3c. Lyse cells

Add 200  $\mu\text{L}$  of Lysis Solution C (**B8803**) to the sample; vortex thoroughly (15 seconds). A homogeneous mixture is essential for efficient lysis. Continue with step 4.

## D. Whole Blood Preparation

#### 1d. Collect blood

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

#### 2d. Prepare blood

Place 20  $\mu\text{L}$  of the Proteinase K solution into a 1.5 mL microcentrifuge tube. Add up to 200  $\mu\text{L}$  of the whole blood sample to the tube. If the sample is less than 200  $\mu\text{L}$ , add the Resuspension Solution to bring the volume up to 200  $\mu\text{L}$ .

**Note:** If the sample is already dispensed in a tube, the Proteinase K solution can be added to the sample. Vortex to ensure thorough mixing of the enzyme. Whole blood can be stored at 4 °C for several hours before preparing DNA. If residual RNA is not a concern, continue with step 3d.

**Optional RNase A treatment:** If RNA-free genomic DNA is required, add 20  $\mu\text{L}$  of RNase A Solution and incubate for 2 minutes at room temperature; continue with step 3d.

#### 3d. Lyse cells

Add 200  $\mu\text{L}$  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. Continue with step 4.

### E. White Blood Cell (WBC) Preparation

#### 1e. Prepare white blood cells from whole blood

Prepare WBCs from 500  $\mu\text{L}$  of whole blood per preparation; see Appendix for an ammonium chloride lysis procedure.

#### 2e. Resuspend cells

Resuspend the pellet thoroughly in 200  $\mu\text{L}$  of the Resuspension Solution; add 20  $\mu\text{L}$  of the Proteinase K solution to the sample and vortex briefly to ensure thorough mixing of the enzyme. If residual RNA is not a concern, continue with step 3e.

**Optional RNase A treatment:** If RNA-free genomic DNA is required, add 20  $\mu\text{L}$  of RNase A Solution and incubate for 2 minutes at room temperature, then continue with step 3e.

#### 3e. Lyse cells

Add 200  $\mu\text{L}$  of Lysis Solution C (**B8803**) to the sample; vortex thoroughly. A homogeneous mixture is essential for efficient lysis. Incubate at 55 °C for 10 minutes. Continue with step 4.

This is a continuation of the procedure from the samples prepared in Sections A, B, C, D, and E.

### DNA Isolation From All Listed Sample Types

#### 4. Column preparation

Add 500  $\mu\text{L}$  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  $12,000 \times g$  for 1 minute. Discard 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  $\mu\text{L}$  of ethanol (95–100%) to the lysate; mix thoroughly by vortexing 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 the DNA when transferring contents into the binding column. Centrifuge at  $\geq 6500 \times g$  for 1 minute. Discard the collection tube containing the flowthrough liquid and place the binding column in a new 2 mL collection tube.

#### 7. First wash

Prior to first use, dilute the Wash Solution Concentrate with ethanol as described under Preparation Instructions. Add 500  $\mu\text{L}$  of Wash Solution to the binding column and centrifuge for 1 minute at  $\geq 6,500 \times g$ . Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 mL collection tube.

#### 8. Second wash

Add another 500  $\mu\text{L}$  of Wash Solution to the binding column; centrifuge for **3 minutes** at maximum speed ( $12,000\text{--}16,000 \times g$ ) to dry the binding column. The binding column must be free of ethanol before eluting the DNA. Centrifuge the column for one additional 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 flowthrough liquid and place the binding column in a new 2 mL collection tube.

#### 9. Elute DNA

Pipette 200  $\mu\text{L}$  of the Elution Solution directly into the center of the binding column; centrifuge for 1 minute at  $\geq 6,500 \times g$  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  $\mu\text{L}$  of Elution Solution and eluting into a new 2 mL collection tube (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 DNA, 2–8 °C is recommended. For long-term storage of DNA, –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 Mammalian Genomic DNA Kit is designed so that the DNA always remains in solution, thus avoiding resuspension issues. However, if you find it necessary to concentrate the DNA, ethanol precipitation in the presence of sodium acetate is recommended.<sup>1</sup>

## Results

The concentration and quality of the genomic DNA prepared with the GenElute kit 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 and 280 nm using a quartz microcuvette. The absorbance should be between 0.1 and 1.0 (or within the linear range of your spectrophotometer). An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/mL of doublestranded DNA. The ratio of absorbance at 260 nm to 280 nm ( $A_{260}/A_{280}$ ) should be 1.6–1.9. The size and quality of the DNA can be determined by agarose gel electrophoresis.<sup>1</sup> A gel containing 0.8% agarose (Catalog No. **A9539**) in 0.5X TBE Buffer (Catalog No. **T6400**) works well for the resolution of genomic DNA. The DNA can be visualized by staining with an intercalating dye such as ethidium bromide (Catalog No. **E1510**) and measured against a known DNA marker such as Lambda DNA *EcoR* I *Hind* III digest (Catalog No. **D9281**). 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.<sup>2</sup>

## Other Related Products

- Agarose (**A9539**)
- Ethanol, 95+% (**E7148**)
- Ethanol, 100% (**E7023**)
- Ethanol, absolute (**459836**)
- Tris-EDTA Buffer (100X) (**T9285**)
- Ethidium Bromide, 10 mg/mL (**E1510**)
- Microcentrifuge Tubes, 1.5 mL (**T9661**)
- *Taq* DNA Polymerase (**D1806, D4545**)
- AccuTaq™ LA DNA Polymerase (**D8045**)
- KlenTaq LA DNA Polymerase Mix (**D5062**)
- Long PCR Core Kit (**LCOR1**)
- Deoxynucleotide (dNTP) Mix, 10 mM (**D7295**)
- Lambda DNA *EcoR* I *Hind* III digest (**D9281**)
- Gel Loading Solution (**G2526**)
- *EcoR* I (40,000 units/mL) (**R4640**)
- Ammonium Chloride (**A9434**)
- Sodium Bicarbonate (**S7277**)
- TBE Buffer, 5X Concentrate (**T6400**)
- Water, Molecular Biology Reagent (**W4502**)

## 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)

## Troubleshooting Guide

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*Binding column is clogged*

**Cause** — Sample is too large.

**Solution** — In the future, use fewer cells, smaller pieces of tissue, smaller pieces of rodent tail, or a smaller quantity of whole blood. To salvage the current preparation, increase the *g*-force and/or spin longer until lysate passes through the binding column. The yield of genomic DNA may be reduced.

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**Cause** — Tissue or rodent tail is inefficiently disrupted.

**Solution** — Extend the Proteinase K digestion at 55 °C. To expedite lysis, cut tissue or rodent tail into smaller pieces and mix frequently during digestion to ensure more efficient lysis. Inverting the sample tube after Proteinase K digestion will ensure a more homogeneous mixture. Do not use a combined solution (Proteinase K + Lysis Solution) that has been stored.

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*Low yield of genomic DNA*

**Cause** — Sample may be old or degraded OR Tissue is inefficiently disrupted.

**Solution** — The yield will vary among different types of cells, tissues, and fresh whole blood. Use cultures before they reach maximum density or as they become fully confluent. Harvest tissues or rodent tails as rapidly as possible. Use whole blood within a few hours. If samples are being stored for future use, flash-freeze the cells or tissue in liquid nitrogen. Whole blood should be kept at 4 °C no longer than 12 hours.

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**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.

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**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 be performed.

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**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.

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**Cause** — The eluate contains residual ethanol from the wash solution.

**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 the DNA.

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**Cause** — Wash Solution Concentrate was not diluted before use.

**Solution** — Check that the Wash Solution Concentrate was properly diluted with ethanol before use.

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*Low yield of genomic DNA*

**Cause** — DNA was eluted with water 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.

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*Purity of the DNA is lower than expected;  $A_{260}/A_{280}$  ratio is too low*

**Cause** — 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.

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**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 the absorbance readings.

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*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.

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*DNA is sheared*

**Cause** — Genomic DNA was handled improperly.

**Solution** — This kit was designed to eliminate DNA precipitation and common resuspension 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.

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**Cause** — Sample is old, degraded, or has undergone repeated freeze/thaw cycles.

**Solution** — Old starting material may yield degraded DNA in the eluate. Fresh cell and tissue, rodent tail, and whole blood preparations should be used immediately. Alternatively, cells and tissue can be frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until needed. Rodent tails can be stored at  $-20^{\circ}\text{C}$  for several weeks or  $-70^{\circ}\text{C}$  for several months. Whole blood can be stored at  $4^{\circ}\text{C}$  for up to 12 hours.

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*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, if necessary, after emptying the collection tube, for an additional 1 minute at maximum speed (12,000–16,000  $\times g$ ).

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**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 Wash Solution in step 8.

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## Appendix: Ammonium Chloride Lysis Procedure for Isolating White Blood Cells from Whole Blood

1. Collect whole blood in an anticoagulant tube.
2. Add 500  $\mu\text{L}$  of whole blood to 1 mL of ammonium chloride lysis solution\*. Gently mix (by inversion or on a table rocker) at room temperature for 5 minutes, then spin for 5 minutes at  $700 \times g$  in a microcentrifuge.
3. Discard supernatant. Gently resuspend pellet in one additional mL of ammonium chloride lysis solution; mix gently and spin as in step 2. Discard the supernatant, keep the cell pellet on ice and resume the White Blood Cell Protocol (1e).

**\*Ammonium Chloride Lysis Solution:**

160 mM ammonium chloride (Catalog No. **A9434**), 20 mM sodium bicarbonate (Catalog No. **S7277**); pH to 7.2 with HCl.

# Experienced User Protocol

## 1 Release DNA from Cultured Cells, Tissues (including rodent tails), Fresh Whole Blood or White Blood Cells

- As described on pages 4–6

### For All Starting Materials:

## 2 Prepare Column

- Add 500  $\mu\text{L}$  Column Preparation Solution to binding column.
- Spin at  $\geq 12,000 \times g$ , 1 min. Discard flow-through.

## 3 Bind DNA to Column

- Add 200  $\mu\text{L}$  ethanol to lysed cells or tissue. Vortex or invert to mix.
- Transfer to binding column. Spin  $\geq 6500 \times g$ , 1 min.

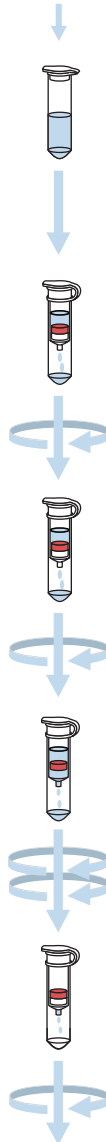
## 4 Wash to Remove Contaminants

- Transfer column to new collection tube. Add 500  $\mu\text{L}$  Wash Solution to column. Spin at  $\geq 6500 \times g$ , 1 min.  
**Note:** Ethanol must be added to Wash Solution concentrate before first use.
- Transfer column to new collection tube. Add second 500  $\mu\text{L}$  Wash Solution to column.
- Spin at  $\geq 12,000 \times g$  for 3 min. to dry column.

## 5 Elute Purified DNA

- Transfer column to new collection tube.
- Add 200  $\mu\text{L}$  Elution Solution. Spin at  $\geq 6500 \times g$ , 1 min.
- Optional: Repeat elution in same or new tube.

## Starting Material



## Pure Mammalian Genomic DNA

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