VIOGENE

Cat. No.: GG1001 (50 preps/Kit)
GG1002 (250 preps/Kit)

Blood & Tissue Genomic DNA Extraction Miniprep System

For research use only

Service

Viogene regards it very important to provide satisfactory service to our every customer. In order to guarantee the best quality of our products, we value our customers' comments and suggestions on our services, or the performance, new applications, and techniques of our products. If there is any question or comment concerning the use of our products, please do not hesitate to contact our Technical Service Department by phone, e-mail, or fax, or to contact your local sales representatives. Our experienced staffs and researchers are pleased to provide you with technical help and advice. If you have problems on attaining the expected performance with our products, please contact our Technical Service Department for technical advice. If any product fails to perform properly not due to incorrect handling, please contact us or your local sales representatives for assistance.

Quality Control

We strictly require good quality control of our products by regular testing of each lot to maintain a satisfactory yield of DNA or RNA. Testing results of all lots of each product are documented. Any inquiry to access them is welcome.

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Description

Viogene Blood & Tissue Genomic DNA Extraction Miniprep System provides a fast and efficient method to purify genomic DNA from various samples such as cultured animal cells, animal tissues, whole blood, buffy coat, lymphocytes, plasma, serum, bacteria, yeasts, DNA virus, paraffin-embedded tissues, etc. Without need of time-consuming phenol/chloroform extraction and ethanol precipitation, this simple spin-column method can isolate genomic DNA of predominantly 20-30 kb free from protein and salt contaminants.

Sample	Maximum Amount		
Whole Blood	200 µl	Up to 10*	50 min
Animal Cells	10^7	Up to 100	50 min
Animal Tissue	30 mg	Up to 100*	1.5 hr
Mouse Tail	0.5 cm	Up to 15	1.5-5 hr
Bacteria/Yeasts	$10^9/10^8$	Up to 80	1.5 hr
Paraffin-Embedded Tissue	25 mg (including paraffin)	Up to 100*	2 hr

* Yield depends on individuals or types.

Downstream Applications

* PCR
* Restriction Analysis
* Southern Blotting

Product Contents

	GG1001 (50 preps) (Cat. No.)	GG1002 (250 preps) (Cat. No.)
LYS Buffer	12 ml (GG1001S01)	60 ml (GG1002S01)
EX Buffer	13 ml (GG1001S02)	60 ml (GG1002S02)
WS Buffer	15 ml* (001001SWS)	45 ml x 2** (001002SWS)
Proteinase K	10 mg*** (GG1001E01)	10 mg x 5*** (GG1002E01)
B/T Genomic DNA Mini Column	50 pieces	250 pieces
Collection Tube	100 pieces	500 pieces
Protocol	1	1

* For GG1001 (**50 preps**), add 60 ml of 98-100% ethanol into WS Buffer bottle when first open.

** For GG1002 (**250 preps**), add 180 ml of 98-100% ethanol into each WS Buffer bottle when first open.

*** Add 1 ml sterile ddH_2O to reconstitute one tube of the provided Proteinase K by vortexing for 1 minute. Make sure that Proteinase K has been completely dissolved. The solution should look clear. The concentration of the Proteinase K stock solution is 10 mg/ml. Store the solution at 4℃.

Buffers and Proteinase K are available for separate purchase. Please refer to the Cat. No. listed above for ordering.

Shipping and Storage

All components of Viogene Blood & Tissue Genomic DNA Extraction Miniprep System are stable at room temperature (20-25℃) for one year.

Important Notes

Please read the following notes before starting the procedures.

1. Buffers in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn to protect from skin contact.

2. All procedure should be done at room temperature (20-25℃).

3. Prepare a 60℃ and/or 70℃ water bath or incubator.

4. Add 1 ml sterile ddH_2O to reconstitute the provided Proteinase K by vortexing. Make sure that Proteinase K has been completely dissolved. The solution should look clear. The concentration of the Proteinase K stock solution is 10 mg/ml. Store the solution at 4℃.

5. Do **not** add and keep Proteinase K directly in EX Buffer.

6. For GG1001 (**50** preps), add 60 ml of 98-100% ethanol into WS Buffer bottle **when first open**. For GG1002 (**250** preps), add 180 ml of ethanol into each WS Buffer bottle **when first open**. Ethanol is provided by the user.

7. Centrifuge steps done at full speed refers to 10,000 x *g* or 13,000-14,000 rpm of a microcentrifuge.

8. Do **not** use more than the suggested maximum amount of sample (refer to **Viogene's Hints**, No. 1, page 21).

9. Homogenization of the tissue sample can greatly **reduce** the time of sample lysis.

10. RNA may be copurified with genomic DNA. RNA will not affect PCR, but may affect certain downstream applications. If RNA-free genomic DNA is desired, add RNase A to the sample as indicated in the protocol.

11. After each vortexing step, when the tube is opened, **briefly centrifuge** the tube to bring down the sample attached inside the cap to avoid generation of aerosols and contact with sample.

12. When sample or buffer is added into the column, avoid touching the rim. This is to prevent cross contamination of samples when handling the columns.

13. DNA can be eluted in 10 mM Tris-HCl (pH 9.0), Milli-Q or double-distilled H_2O, or TE buffer (pH 8.0). Since genomic DNA elution takes place most effectively at **pH 9**, to ensure optimal elution, make sure that pH of these elution solutions are between 8.0 and 9.0.

Protocol

Please refer to the Table of Contents on page 4 to choose the appropriate protocol according to the kind of sample used.

I. Blood Protocol

For samples including whole blood (anti-coagulant added), buffy coat, serum, plasma, body fluid, 10^6-10^7 lymphocytes and cultured cells in 200 µl PBS.

1. Pipet up to 200 µl sample into a 1.5-ml sterile eppendorf tube. When the sample volume is less than 200 µl, add PBS to make up to 200 µl.

   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample at this step.

2. Add 20 µl Proteinase K and 200 µl EX Buffer into the sample. Mix immediately by vortexing for 20 seconds.

   Do NOT add and keep Proteinase K directly in EX Buffer.

   When sample volume is larger than 200 µl, increase the amount of Proteinase K and EX Buffer proportionally.

3. Incubate at 60℃ for 20 minutes to lyse the sample. Vortex or invert mix the sample **every 3-5 minutes** during incubation.

   Ensure complete sample lysis:
   whole blood sample should NOT appear viscous;
   buffy coat should NOT contain insoluble residues;
   cell sample should appear translucent.

4. Adjust the incubator to 70℃ to incubate for 20 minutes.

   Alternatively, place the sample to another 70℃ incubator and incubate for 10 minutes.

5. Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70℃ (500 µl/prep) for DNA elution at Step 10.

   Refer to **Important Notes**, No. 13, page 8, and **Viogene's Hints**, No. 3, page 22, for the choice of elution solution.

6. Add 210 µl of absolute ethanol or isopropanol to the sample from Step 4 and mix by vortexing.

If the sample volume is more than 200 µl, increase the amount of ethanol or isopropanol proportionally.

7. Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipette all the mixture (including any precipitate) into the column without touching the rim. Centrifuge at 8,000 rpm (6,000 x *g*) for 2 minutes. Place the column onto a **new** Collection Tube.

If a precipitate formed in Step 6, apply both the precipitate and mixture into the column.

8. Wash the column **twice** with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x *g*) for 2 minutes. Discard the flow-through after centrifugation.

Ensure that ethanol has been added into WS Buffer bottle when first open.

9. Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.

Refer to **Important Notes**, No. 7, page 7.

10. Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 µl of the preheated elution solution from Step 5.

Refer to **Viogene's Hints**, No. 5, page 22, for optimal DNA elution.

11. Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.

12. Store eluted DNA at 4℃ or -20℃.

Store DNA at 4℃ for frequent use or at -20℃ for long-term storage. Repeated freeze-thaw cycles can cause shearing of genomic DNA.

II. Tissue Protocol

1. Cut 30 mg of tissue (15 mg spleen) into small pieces and place the sample into a 1.5-ml sterile eppendorf tube. Add 200 µl LYS Buffer and homogenize the sample.

   If the sample size is larger than 30 mg, increase the amount of LYS Buffer proportionally.

2. Add 20 µl Proteinase K to the sample. Mix immediately by vortexing for 20 seconds.

   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample.

3. Incubate at 60℃ for 1 hour to lyse the sample. If tissue is difficult to lyse, increase the incubation time to 2-3 hours. Vortex or invert mix the sample **every 10-15 minutes**.

   Vortex-mixing is important for complete breaking up and digesting of the tissue.

   Ensure complete sample lysis; sample after complete lysis should appear translucent.

4. Adjust the incubator to 70℃ to incubate for 20 minutes.

   Alternatively, place the sample to another 70℃ incubator and incubate for 10 minutes.

5. Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH_2O, or TE buffer (provided by user) at 70℃ (500 µl/prep) for DNA elution at Step 11.

   Refer to **Important Notes**, No. 13, page 8, and **Viogene's Hints**, No. 3, page 22, for the choice of elution solution.

6. Add 200 µl of EX Buffer to the sample, mix by vortexing and incubate at 70℃ for 10 minutes.

   If the sample contains undigested remains after incubation, centrifuge for 5 minutes at full speed and use only the supernatant in the following steps.

7. Add 210 µl of absolute ethanol or isopropanol to the sample and mix by vortexing.

If the sample mixture is more than 400 µl, increase the amount of ethanol or isopropanol proportionally.

8. Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipette all the mixture (including any precipitate) into the column without touching the rim. Centrifuge at 8,000 rpm (6,000 x *g*) for 2 minutes. Place the column onto a **new** Collection Tube.

If a precipitate formed in Step 7, apply both the precipitate and mixture into the column.

9. Wash the column **twice** with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x *g*) for 2 minutes. Discard the flow-through after centrifugation.

Ensure that ethanol has been added into WS Buffer bottle when first open.

10. Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.

Refer to **Important Notes**, No. 7, page 7.

11. Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 µl of the preheated elution solution from Step 5.

12. Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.

13. Store eluted DNA at 4℃ or -20℃.

Store DNA at 4℃ for frequent use or at -20℃ for long-term storage. Repeated freeze-thaw cycles can cause shearing of genomic DNA.

III. Mouse Tail Protocol

1. Cut into **small** pieces of a segment of mouse tail of up to 0.5 cm. Place the sample into a 1.5-ml sterile tube.

   Segment close to the **tail tip** is preferred. Segment away from the tip is thicker and takes longer time to lyse completely.

2. Add 20 µl Proteinase K and 200 µl LYS Buffer to the sample. Mix immediately by vortexing for 20 seconds.

   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample.

   Further addition of 20 µl of 10 mg/ml **Proteinase E** (DNase-free) (provided by user) can enhance mouse tail lysis and increase DNA yield.

3. Incubate at 60℃ for 1-4 hours or overnight to lyse the tail tissue. Vortex or invert mix the sample **every 20-30 minutes** during incubation.

   Ensure complete sample lysis; sample after complete lysis should appear translucent with only hair and bone residues remained.

4. Follow the **Tissue Protocol** starting from Step 4 on Page 11.

IV. Paraffin-Embedded Tissue Protocol

1. Cut a small section of paraffin-embedded tissue (about 25 mg) and put into a 1.5-ml eppendorf tube.

2. Add 1 ml xylene and incubate at room temperature with occasional mixing for 30 minutes to extract paraffin from tissue.

3. Centrifuge at full speed for 5 minutes. Remove the supernatant by pipetting.

Refer to **Important Notes**, No. 7, page 7.

4. Add 1 ml absolute ethanol to the tissue pellet, mix, and centrifuge at full speed for 5 minutes. Remove ethanol-containing xylene residue by pipetting.

5. Evaporate ethanol residue by incubating at 37℃ for 10 minutes.

6. Resuspend the pellet in 200 µl LYS Buffer.

7. Follow the **Tissue Protocol** starting from Step 2 on page 11.

V. Bacteria Protocol

A. Bacteria

1. Pellet log-phase grown bacteria of up to 10^9 (or up to 3 ml culture) at 7,500 rpm (5,000 x *g*) for 10 minutes.

2. Resuspend the pellet in 200 µl lysozyme reaction solution (20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 20 mg/ml lysozyme). Incubate at 37℃ for 30 minutes.

If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the resuspended cells.

Lysozyme is provided by user.

3. Add 20 µl Proteinase K and 200 µl EX Buffer to the sample. Mix immediately by vortexing for 20 seconds.

Do NOT keep Proteinase K directly in EX Buffer.

4. Incubate at 60℃ for 30 minutes to lyse the bacterial cells. Vortex or invert mix the sample **every 5 minutes** during incubation.

Incubation with mixing facilitates lysis. Ensure complete cell lysis; sample after complete lysis should appear translucent.

5. Adjust the incubator to 70℃ to incubate for 30 minutes.

6. Follow the **Blood Protocol** starting from Step 5 on Page 9.

B. Bacteria in biological fluids

1. Pellet cells by centrifuging at 7,500 rpm (5,000 x *g*) for 10 minutes.

If RNA-free genomic DNA is desired, add 5 µl of 50 mg/ml RNase A to the resuspended cells.

2. Resuspend the pellet in 200 µl LYS Buffer.

3. Follow the **Tissue Protocol** starting from Step 2 on page 11.

C. Bacteria from eye, nasal, or pharygeal swabs

1. Collect bacterial cells by rinsing and soaking the swabs in 2 ml PBS at room temperature for 2-3 hours.

2. Pellet cells by centrifuging at 7,500 rpm (5,000 x *g*) for 10 minutes.

3. Resuspend the pellet in 200 µl LYS Buffer.

If RNA-free genomic DNA is desired, add 5 µl of 50 mg/ml RNase A to the resuspended cells.

4. Follow the **Tissue Protocol** starting from Step 2 on page 11.

VI. Yeast Protocol

1. Pellet log-phase grown yeast cells up to 10^8 (or up to 3 ml culture) at 7,500 rpm (5,000 x *g*) for 10 minutes.
2. Resuspend the pellet in 500 µl sorbitol reaction solution (1 M sorbitol; 100 mM EDTA; 14 mM β-mercaptoethanol; 200 U lyticase or zymolase).

If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the resuspended cells.

Lyticase or zymolase is provided by user.

3. Incubate at 30℃ for 30 minutes.
4. Centrifuge at 7,500 rpm (5,000 x *g*) for 5 minutes. Resuspend the pellet in 200 µl LYS Buffer.
5. Follow the **Tissue Protocol** starting from Step 2 on page 11.

VII. Virus Protocol

1. Prepare viral DNA from blood or body fluid, the **Blood Protocol** on page 9-10 is suggested.
2. Prepare integrated viral DNA, the **Blood Protocol** on page 9-10 or **Tissue Protocol** on page 11-12 is suggested.

Troubleshooting Guide

Problem	Possible Reason	Solution
Brown color residues remain on the membrane of a column after washing	Incomplete digestion of hemoglobin	Vortex the sample after Proteinase K is added. Mix the sample **every 3-5 minutes** during incubation.
	No alcohol or alcohol of incorrect amount is added to the sample before loaded into the column	Before passing the column, add 210 µl (or suitable volume) of absolute alcohol to the sample.
	WS Buffer does not contain ethanol	Make sure that ethanol is added into the WS Buffer bottle **when first open** (refer to **Important Notes**, No. 6, page 7).
Low or no yield of DNA	Sample contains too low amount of genomic DNA	Increase the sample amount, Proteinase K, and buffer proportionally. If the sample is whole blood, prepare buffy coat from a larger volume of blood.
	Blood or cell sample is not lysed completely	Add another 20 µl fresh Proteinase K per sample and repeat incubation.
	No alcohol or alcohol of incorrect amount is added to the sample before loaded into the column	Before passing the column, add 210 µl (or suitable volume) of absolute alcohol into the sample.

Problem	Possible Reason	Solution
Low or no yield of DNA	Elution solution is not preheated at 70℃	Preheat the elution solution at 70℃ before used.
	pH of the elution solution is too low	Make sure that the pH of 10 mM Tris-HCl, ddH_2O or TE buffer for elution is between 8.0-9.0.
	WS Buffer does not contain ethanol	Make sure that ethanol is added into the WS Buffer bottle **when first open** (refer to **Important Notes**, No. 6, page 7).
Column is clogged when passing the sample	Tissue sample still contains undigested remains after lysis	After Proteinase K digestion, centrifuge the sample at full speed for 5 minutes to remove undigested remains.
	Blood sample contains clots	Use whole blood sample mixed well with anti-coagulant to prevent formation of blood clot. Do **not** use blood clot for genomic DNA extraction.
	Sample is very viscous	Too much sample is used. Reduce the sample amount.
A_{260}/A_{280} ratio of eluted genomic DNA is low	Protein in the sample is not completely degraded	Vortex the sample after Proteinase K is added. Mix the sample at constant intervals during incubation.

Problem	Possible Reason	Solution
A_{260}/A_{280} ratio of eluted genomic DNA is low	Protein in the sample is not completely degraded	Add 20 µl fresh Proteinase K per sample and continue incubation.
	No alcohol or alcohol of incorrect amount is added to the sample before loading into the column	Before passing the column, add 210 µl (or suitable volume) of absolute alcohol into the sample.
	Eluted genomic DNA contains contaminants	Do **not** touch the rim of the column during sample or buffer loading.
	Eluted genomic DNA contains ethanol	After the final wash, centrifuge the column at full speed for another 2 minutes to remove the ethanol residue completely.
	Using ddH_2O of acidic pH (5.0-6.0) to dilute DNA samples for spectrophotometric analysis	Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the DNA samples (refer to **Viogene's Hints**, No. 4, page 22).
A_{260}/A_{280} of eluted genomic DNA is high (>1.9)	Eluted genomic DNA contains a lot of RNA	Add RNase A to the sample as described in the protocol.

Problem	Possible Reason	Solution
Genomic DNA appears smearing and degraded	Sample is not fresh or stored improperly for a long time	Flash freeze fresh samples in liquid nitrogen and store at -80℃ if not used immediately.
	Blood sample is not fresh or stored improperly for a long time	Use fresh blood, or blood stored at room temperature for fewer than 2 days.
	Gel eletrophoresis is performed in used running buffer contaminated with DNase	Use **fresh** TAE or TBE running buffer for electrophoresis.
	Paraffin-embedded tissue is used as sample	Genomic DNA isolated from this kind of sample is usually degraded. It is still suitable for PCR application, but is not recommended for Southern blotting and restriction analysis.

Viogene's Hints

1. Low yield or purity of genomic DNA is usually due to incomplete digestion or lysis of the sample. Starting with a maximum amount or volume of samples does NOT usually give the best yield of DNA. Instead, it always results in incomplete sample lysis and degradation of proteins, thus making extraction of all DNA from the sample unfeasible. Further, it always requires subsequent removal of undigested residues and yields viscous sample lysate. When the lysate is too viscous, it not only has difficulty in passing the column, but also indicates the presence of an abundant amount of contaminants such as proteins and salts. Contaminants of high amount not only affect DNA binding, but also may not be washed off completely, leading to carry over to the eluted genomic DNA. Therefore, a good quality and yield of DNA is only expected when a sample is **completely** digested. We advise starting with half of the maximum amount of sample suggested. When there is no problem in digesting the sample completely and passing the lysate through the column, amount of the sample to be applied can be increased gradually in the subsequent preparations.

2. When buffy coat is used as sample, make sure that cells in it are not more than 1×10^7, otherwise the lysate obtained will be too viscous to pass through the column easily.

3. DNA should not be eluted in ddH_2O for storage because it suffers from gradual degradation through acid hydrolysis. Since DNA is more stable in a slightly alkaline (pH 7.5-9) buffering condition, 10 mM Tris-HCl (pH 9.0) or TE buffer is considered a better choice than water for DNA elution. When TE buffer is used for elution, make sure that EDTA in the buffer does not affect further enzymatic reaction.

4. Using ddH_2O of acidic pH (5.0-6.0) to dilute DNA and RNA samples for spectrophotometric analysis will significantly decrease A_{260}/A_{280} ratio of the sample (Wilfinger et al., 1997). 10 mM Tris-HCl of pH 7.5 or TE buffer should be used to dilute the samples.

5. Elute DNA according to the yield expected. Use 50 µl elution solution for less than 1 µg DNA, 100 µl for less than 5 µg DNA, and 200 µl twice for more than 30 µg DNA. Generally, a higher DNA recovery can be attained by eluting the column twice. That is, eluting twice, e.g., with 100 µl elution solution, yields more DNA in total than eluting once with 200 µl elution solution.

Reference: Wilfinger, W. W., Mackey, K., and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* **22**:474-481.

Viogene Products

Product	Cat. No.	Package Size	Sample	Expected Yield
Mini-M Plasmid	GF1001 GF1002	50 250	1-5 ml culture	up to 20 μg
Midi-V100 Plasmid	GDV1001 GDV1002	25 50	25-100 ml culture	up to 100 μg
Maxi-V500 Plasmid	GMV1001 GMV1002	10 25	100-250 ml culture	up to 500 μg
Blood & Tissue Genomic DNA Mini	GG1001 GG1002	50 250	200 μl whole blood 15-30 mg tissue	up to 10 μg up to 100 μg
Blood Genomic DNA Midi	GGD1001 GGD1002	20 100	1 ml whole blood	up to 50 μg
Blood Genomic DNA Maxi	GGM1001 GGM1002	10 50	5 ml whole blood	up to 300 μg
Plant Genomic DNA Mini	GPG1001 GPG1002	50 250	100 mg tissue	up to 40 μg
Plant Genomic DNA Maxi	GPGM1001	20	1 g tissue	up to 1 mg
Total RNA Mini	GR1001 GR1002	50 250	10-20 mg tissue $1x10^7$ cells	10-45 μg up to 30 μg
Total RNA Midi	GRD1001 GRD1002	10 50	0.1-0.2 g tissue $3-7x10^7$ cells	200-450 μg up to 300 μg
Total RNA Maxi	GRM1001 GRM1002	6 24	0.5-1 g tissue $2-10x10^8$ cells	1-5 mg up to 6 mg
Viral RNA Mini	GVR1001 GVR1002	50 250	150 μl body fluid	up to 90% recovery
Plant Total RNA Mini	GPR1001 GPR1002	50 250	100 mg tissue	up to 100 μg
Plant Total RNA Maxi	GPRM1001	10	1 g tissue	up to 1 mg
Gel-M Gel Extraction	EG1001 EG1002	50 250	50-200 mg agarose gel	50-80% recovery (100 bp-10 kb)
PCR-M Clean Up	PF1001 PF1002	50 250	10-100 μl DNA	up to 95% recovery (100 bp-10 kb)
VioTaq DNA Polymerase	VT1001	500 U (5 U/μl) 10X PCR Buffer containing 20 mM $MgCl_2$		
VioTwinPack Kit	VTP1001	500 U *VioTaq* DNA Polymerase (5 U/μl) 10X PCR Buffer containing 20 mM $MgCl_2$ 40 mM of dNTP mix (10 mM each)		
Clear-band Agarose	AG0050	50 g 100 g		