Ver 3.0

Handbook for

- Blood SV mini
- Blood SV midi
- Blood SV maxi

Exgene™ Blood SV

DNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

Contact Information

www.geneall.com

Tel : 82-2-407-0096

Fax : 82-2-407-0779

Sales Email : sales@geneall.com

Technical Information Email : tech@geneall.com

Visit GeneAll® Community

www.geneall.com

Trademarks

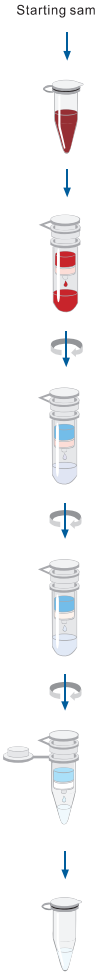
Hybrid-Q™, Exprep™, Exfection™, Expin™, Exgene™, *GenEx™*, DirEx™, Hybrid-R™, RiboEx™, Ribospin™, Riboclear™, Allspin™, RiboSaver™, EzClear™, EzSep™, EzPure™, EzPass™, AmpONE™, AmpMaster™, RealAmp™, HyperScript™, ProtinEx™, PAGESTA™, STEADi™, GENTi™, GENTi ™ Advanced, SolMate™, and AllEx® are trademarks of GeneAll Biotechnology Co., Ltd.

This protocol handbook is included in :

GeneAll® Exgene™ Blood SV mini (105-101, 105-152)
GeneAll® Exgene™ Blood SV midi (105-201, 105-226)
GeneAll® Exgene™ Blood SV maxi (105-310, 105-326)

Visit www.geneall.com for FAQ, Q&A and more information.

Brief protocol (mini)
for Blood/Cultured Cells/Buffy coat/Plasma/Serum



Lyse

1. Transfer the sample to a micro centrifuge tube
2. (Optional) Add 20 μl of RNase A solution and incubate for 2 min at RT
3. Add 20 μl of Proteinase K solution and 200 μl of Buffer JBL
4. Incubate for 10 min at 56°C

Bind

5. Add 200 μl of Absolute Ethanol
6. Transfer the mixture into a mini column
7. Centrifuge for 1 min at 13,000 rpm

1st Wash

8. Add 500 μl of Buffer JBW into the mini column
9. Centrifuge for 1 min at 13,000 rpm
10. Repeat step 8-9

2nd Wash

11. Add 700 μl of Buffer NW into the mini column
12. Centrifuge for 1 min at 13,000 rpm
13. Perform an additional centrifuge for 3 min at ≥13,000 rpm

Elute

14. Apply 50-100 μl of Nuclease free water into the mini column
15. Incubate for 2 min at room temperature
16. Centrifuge for 2 min at 13,000 rpm

Pure DNA

INDEX

Protocols

Kit Contents

Cat. No.	105-101	105-152	Storage
Type	mini		
Components	Quantity		
No. of preparation	100	250	Room temperature (15~25°C)
Mini Column Type G (with collection tube)	100	250	
2 ml Collection tube	200	500	
Buffer JBL	25 ml	60 ml	
Buffer JBW (concentrate) *	54 ml	63 ml (2 ea)	
Buffer NW (concentrate) *	25 ml	50 ml	
Nuclease free water	15 ml	30 ml	
PK Storage buffer	4 ml	7 ml	
Proteinase K **	48 mg	120 mg	
Protocol handbook	1	1	

** Before the first use, add the appropriate amount of absolute ethanol (ACS grade quality or higher) to Buffer JBW, NW as indicated on the bottle.*

*** For the long-term storage of Proteinase K, store at 4 °C. But after reconstitution of Proteinase K, store at -20 °C. Refer to the instruction of Proteinase K on page 9.*

Materials to Be Supplied by the User

- Reagent : Absolute ethanol (ACS grade quality or higher), 1 M DTT, 10% Sodium Dodecyl Sulfate
- Disposable material : Sterile pipette tips, Disposable gloves, Sterile micro centrifuge tubes
- Equipment : Micro centrifuge, Vortex mixer, Heat block, Suitable protector

Product Specifications

Exgene™ Blood SV mini	
Type	Spin
Maximum amount of starting samples	Liquid sample : 200 µl/prep Cultured cell : 5 x 10^6/prep Dried blood spot : 5 mm (1/5") punched sample 3 ea/prep
Preparation time	≥30 min
Maximum loading volume of mini column	750 µl
Minimum elution volume	50 µl

Kit Contents

Cat. No.	105-226	105-201	105-310	105-326
Type	midi		maxi	
Components	Quantity		Quantity	
No. of preparation	26	100	10	26
Column	26	100	10	26
Tube	26	100	10	26
Buffer JBL	80 ml	150 ml (2 ea)	150 ml	150 ml (2 ea)+25 ml
Buffer JBW (concentrate) *	54 ml	99 ml (2 ea)	54 ml	63 ml (2 ea)
Buffer NW (concentrate) *	25 ml	50 ml (2 ea)	25 ml	50 ml+6 ml
Nuclease free water	15 ml	30 ml (2 ea)	15 ml	30 ml
PK Storage buffer	4 ml	7 ml (2 ea)	4 ml (2 ea)	7 ml (2 ea)
Proteinase K **	48 mg	120 mg (2 ea)	48 mg (2 ea)	120 mg (2 ea)
Protocol handbook	1	1	1	1

** Before the first use, add the appropriate amount of absolute ethanol (ACS grade quality or higher) to Buffer JBW, NW as indicated on the bottle.*

*** For the long-term storage of Proteinase K, store at 4 °C. But after reconstitution of Proteinase K, store at -20 °C. Refer to the instruction of Proteinase K on page 9.*

Materials to Be Supplied by the User

- Reagent : Absolute ethanol (ACS grade quality or higher)
- Disposable material : Sterile pipette tips, Disposable gloves, Sterile centrifuge tubes, Serological pipette
- Equipment : Swing bucket centrifuge, Vortex mixer, Heat block or Dry oven, Suitable protector, Pipet aid

Product Specifications

Exgene™ Blood SV midi/maxi	
Type	Spin
Amount of starting samples	Midi prep : 2 ml/prep Maxi prep : 10 ml/prep
Preparation time	≥60 min
Maximum loading volume of column (midi/maxi)	4 ml / 20 ml
Minimum elution volume (midi/maxi)	100 μl / 300 μl

Quality Control

All components in the Exgene™ Blood SV are manufactured and maintained in a state of strict cleanliness.

Rigorous quality control is performed consistently across batches, and only the kits meeting the required standards are authorized for delivery.

Storage Conditions

All components of Exgene™ Blood SV should be stored at room temperature (15~25°C) and protected from direct sunlight exposure.

During shipment or storage under cool ambient conditions, a precipitate may form in Buffer JBL or JBW. In such a case, incubate the bottle at 56°C to dissolve precipitates before use.

Using precipitated buffers will lead to poor DNA recovery. Exgene™ Blood SV is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in Exgene™ Blood SV contain irritants that can be harmful upon contact with skin or eyes, inhalation or ingestion. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions.

Buffer JBL and JBW contains chaotropic agents, which can form highly reactive compounds when combined with bleach.

DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

Exgene™ Blood SV is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Preventing Contamination

Proper microbiological, aseptic technique should always be used when working with trace or evidentiary materials.

Always wear disposable gloves while handling reagents and samples. The use of sterile tip, tube and other instruments is recommended throughout the procedure.

Proteinase K

Exgene™ Blood SV contains Proteinase K to maximize recovery and yield from a variety of sample types.

Add PK-Storage buffer to one tube of lyophilized Proteinase K, and gently invert to dissolve.

Store Proteinase K Solution at 4°C. For storage longer than one year, we suggest storing it at -20°C.

Product Description

It utilizes advanced silica-binding technology to effectively extract pure genomic DNA suitable for various applications.

Initially, different samples are lysed in a specialized buffer containing detergent and lytic enzyme. During optimal binding conditions, DNA from the lysate binds to a silica membrane, while impurities are filtered out into a separate collection tube. Subsequently, the membranes undergo a series of alcohol-based washes to eliminate proteins, cellular debris, and salts. Finally, the purified DNA is eluted into a clean micro centrifuge tube using deionized water or a low ionic strength buffer.

This purified DNA is ready for direct use in PCR, qRT-PCR, or any downstream application.

Intended Use

The Exgene™ Blood SV is designed to extract high-quality DNA from various blood samples, including fresh or frozen whole blood, dried blood spot, serum, plasma, buffy coat, body fluids, cultured cells and swabs.

Using Swinging-bucket Centrifuge in midi/maxi procedures

Exgene™ midi and maxi procedures require the conventional centrifuge which has a swinging-bucket rotor and ability of 4,000~5,000 x g. Use of fixed-angle rotor will cause nonuniform contact between column membrane and solutions, followed by inconsistent result. Low g-force will lead to uncomplete removal of ethanol from column membrane and to inadequate eluting. Compatible centrifuges and rotors are listed below, but you can use any other equivalent.

Company	Centrifuge	Rotor
Beckman CoulterInc. (California, USA)	Allegra X-15R Allegra 25R	Sx4750 Sx4750A TS-5.1-500
Eppendorf AG (Hamburg, Germany)	5804/5804R 5810/5810R	A-4-44
EYELA Inc. (Tokyo, Japan)	5800 5900	RS-410 RS-410M
Hanil Science Industrial Inc. (Incheon, Korea)	Union 5KR Union 55R MF-550 HA1000-6 HA1000-3	R-WS1000-6B W-WS750-6B HSR-4S WHSR-4S
Hettich AG (Kirchlengern, Germany)	Rotina 35 Rotanta 460 Rotixa 50S	1717 1724 5624

PROTOCOL FOR

Whole blood / Body fluid / Cultured cell / Buffy coat

Before experiment

- *Before first use, add absolute ethanol (ACS grade or higher) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare 1.5 ml micro centrifuge tube.*
- *Prepare and store absolute ethanol in the freezer.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C before use.*

1. **Add 20 μl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml micro centrifuge tube (not provided).**
   If the sample volume is larger than 200 μl, increase the amount of Proteinase K proportionally. When the concentration of cells is low, up to 400 μl of starting sample can be used. For 400 μl of sample volume, 40 μl of Proteinase K solution is needed.

2. **Transfer 200 μl of sample to the tube. Use the starting sample listed below.**
   If the sample volume is less than 200 μl, adjust the volume to 200 μl with 1X PBS.

Sample	Max. amount per prep	Preparation
Mammalian whole blood	200 μl	Direct use
Body fluid	200 μl	Direct use
Buffy coat	200 μl	Direct use
Nucleated blood of bird, fish, reptile and amphibian	10 μl	10 μl blood with 190 μl of 1X PBS
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 μl of 1X PBS
Virus	200 μl	200 μl of virus-containing media

3. ***(Optional :)* If RNA-free DNA is required, add 20 μl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided) to the sample, mix by pipetting, and incubate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be co-purified with DNA. RNA can inhibit some down-stream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 μl of Buffer JBL to the tube. Vortex the tube to mix thoroughly. Incubate at 56˚C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   If the sample volume is larger than 200 μl, increase the volume of Buffer JBL in proportion. Ratio of Buffer JBL to the starting sample volume is 1:1.

5. **Add 200 μl of chilled absolute ethanol (not provided) to the sample, vortex 30 sec to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   If the sample volume is larger than 200 μl, increase the ethanol volume proportionally.

6. **Transfer the mixture to the Column Type G (mini) carefully, centrifuge at 13,000 rpm for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**
   If starting sample volume is larger than 200 μl, apply the mixture twice; apply 700 μl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the mini column.

7. **Add 500 μl of Buffer JBW, centrifuge at 13,000 rpm for 1 min, and discard the pass-through and reinsert the mini column back into the collection tube.**

8. **Repeat step 7.**

9. **Apply 700 μl of Buffer NW. Centrifuge at 13,000 rpm for 1 min. Replace the collection tube with new one (provided).**
   Centrifugation at full speed will not affect DNA recovery.

10. **Centrifuge at full speed for 3 min to remove residual wash buffer. Place the mini column in a fresh 1.5 ml micro centrifuge tube (not provided).**
    Care must be taken at this step for eliminating the carryover of Buffer NW. If a carryover of Buffer NW still occurs, centrifuge again at full speed for 1 min with the collection tube before transferring to a new 1.5 ml micro centrifuge tube. Centrifugation must be performed at full speed.

11. **Add 50-100 μl of Nuclease free water to the membrane. Incubate for 2 min at room temperature.**

12. **Centrifuge for 2 min (full speed, RT).**

PROTOCOL FOR
Plasma / Serum

Before experiment

- *Before first use, add absolute ethanol (ACS grade or higher) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare 1.5 ml micro centrifuge tube.*
- *Prepare and store absolute ethanol in the freezer.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C before use.*

1. **Transfer 1 ml of sample to a 1.5 ml micro centrifuge tube (not provided).** The amount of degraded samples should be increased up to 2 ml.

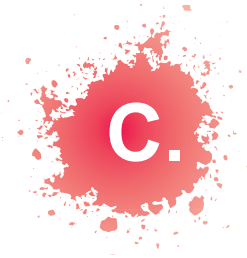
2. **Centrifuge at 13,000 rpm for 5 min. Discard supernatant.**

3. **Add 200 µl of 1X PBS to the tube. Suspend the pellet thoroughly.**

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml micro centrifuge tube (not provided).**

5. **Add 200 µl of Buffer JBL to the tube. Vortex the tube to mix thoroughly. Incubate at 56°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**

6. **Continue with step 5 in A. PROTOCOL FOR BLOOD AND BODY FLUID/ CULTURED CELLS USING MICROCENTRIFUGE on page 13.**



PROTOCOL FOR
Dried blood spot

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare absolute ethanol in the freezer.*
- *Prepare water baths or incubators at 56°C and 70°C.*
- *Prepare 1.5 ml micro centrifuge tube.*
- *All centrifugation should be performed at room temperature.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C before use.*

* This protocol is suitable for blood, both untreated and treated with anticoagulants, which has been spotted and dried on filter paper (Schleicher and Schuell 903 or FTA card).

1. **Place 3~4 punched-out circles from a dried blood spot into a 1.5 ml micro-centrifuge tube (not provided) and add 200 µl of autoclaved distilled water.**
   Use a 5 mm (1/5") single-hole paper puncher to cut out the circles from a dried blood spot.

2. **Add 20 µl of Proteinase K solution (20 mg/ml, provided), vortex to mix, and incubate at 56°C for 30 min. Spin down briefly to remove any drops from inside of the lid.**

3. **Add 200 µl of Buffer JBL and mix thoroughly by vortexing. Incubate at 70°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   It is essential to mix the sample with Buffer JBL completely for efficient lysis. After addition of Buffer JBL, a white precipitate may form. This may disappear during incubation at 70°C and will not affect DNA recovery.

4. **Continue with step 5 in A. PROTOCOL FOR BLOOD AND BODY FLUID/ CULTURED CELLS USING MICROCENTRIFUGE on page 13.**

D.

PROTOCOL FOR
Buccal swab

Before experiment

- *Before first use, add absolute ethanol (ACS grade or higher) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *Prepare and store absolute ethanol in the freezer.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C before use.*

1. **Scrape the swab firmly more than 5~6 times against the inside of cheek.**
   To avoid contamination from other materials, ensure that the person providing the sample has not taken any food or drink in 30 min prior to sample collection.

2. **Place the swab with cut handle using sterile sharp blade or wire cutter in 2 ml micro centrifuge tube (not provided). Add 400 µl of 1X PBS to the tube.**
   Cutters should be rinsed with 70% ethanol to prevent contamination between samples.

3. ***(Optional :)* If RNA-free DNA is required, add 20 µl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided) vortex to mix, and incubate at room temperature for 2 min.**

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 400 µl of Buffer JBL to the sample. Vortex vigorously to mix immediately. Incubate at 56°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**

5. **Add 400 µl of chilled absolute ethanol (not provided) to the lysate, and mix well by vortexing. Briefly spin down to remove any drops from inside the lid.**

6. **Continue with step 6 in A. PROTOCOL FOR BLOOD AND BODY FLUID / CULTURED CELLS USING MICROCENTRIFUGE on page 13.**

PROTOCOL FOR
Saliva and Mouth wash

Before experiment

- *Before first use, add absolute ethanol (ACS grade or higher) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare 1.5 ml micro centrifuge tube.*
- *Prepare and store absolute ethanol in the freezer.*
- *All centrifugation should be performed at room temperature.*
- *Prepare Proteinase K solution (20 mg/ml) for first use.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C before use.*

1. **Collect 10 ml of mouthwash or 1 ml of saliva. For saliva, mix it with 5 ml of 1X PBS and vortex thoroughly.**
   To avoid contamination from other materials, ensure that the person providing the sample has not taken any food or drink in the 30 min prior to sample collection.

2. **Centrifuge at 3,000 rpm for 5 min to pellet cells. Immediately and carefully decant the supernatant. Suspend completely the pellets in 200 µl of 1X PBS.**

3. ***(Optional :)* If RNA-free DNA is required, add 20 µl of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided), vortex to mix, and incu-bate at room temperature for 2 min.**
   Unless RNase A is treated, RNA will be co-purified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 200 µl of Buffer JBL to the sample. Vortex vigorously to mix completely. Incubate at 56°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**

5. **Continue with step 5 in A. PROTOCOL FOR BLOOD AND BODY FLUID/ CULTURED CELLS USING MICROCENTRIFUGE on page 13.**

PROTOCOL FOR
Hair

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare the water bath to 56°C.*
- *Prepare 1.5 ml micro centrifuge tube.*
- *Prepare absolute ethanol in the freezer.*
- *Prepare Buffer H as follow; 10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 2% SDS, 40 mM DTT.*
  (Add DTT immediately before use, because it oxidizes quickly in aqueous solutions.)
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in Buffer JBL and JBW heat to dissolve at 56°C.*

1. **Collect hair root in a 1.5 ml micro centrifuge tube (not provided).**
   The amount of starting sample should not exceed 30 mg. It is recommended to use 2 cm from the root ends of plucked hair samples.

2. **Add 180 µl of prepared Buffer H and 20 µl of Proteinase K solution (20 mg/ml, provided) to the tube, and vortex to mix thoroughly.**

3. **Incubate for 1 hour at 56°C. Spin down briefly to remove any drops from inside of the lid.**

4. **Continue with step 3 in A. PROTOCOL FOR BLOOD AND BODY FLUID/ CULTURED CELLS USING MICROCENTRIFUGE on page 13.**

G. PROTOCOL FOR Sperm

Before experiment

- *Before first use, add absolute ethanol (ACS grade or better) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare the water bath to 56°C.*
- *Prepare 1.5 ml micro centrifuge tube.*
- *Prepare absolute ethanol in the freezer.*
- *Prepare Buffer H2 as follow; 20 mM Tris-HCl (pH 8.0), 20 mM EDTA, 200 mM NaCl, 4% SDS, 80 mM DTT.*
  (Add DTT immediately before use, because it oxidizes quickly in aqueous solutions.)
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in Buffer JBL and JBW heat to dissolve at 56°C.*

1. **Place 100 µl of sperm in a 1.5 ml micro centrifuge tube (not provided). Add 100 µl of Buffer H2 and 20 µl of Proteinase K solution (20 g/ml, provided) to the tube. Mix thoroughly by vortexing.**

2. **Incubate for 1 hour at 56°C. Spin down briefly to remove any drops from inside of the lid.**

3. **Continue with step 3 in A. PROTOCOL FOR BLOOD AND BODY FLUID/ CULTURED CELLS USING MICROCENTRIFUGE on page 13.**

H.

PROTOCOL FOR
2 ml of Whole Blood (midi)

Before experiment

- *Before first use, add absolute ethanol (ACS grade or higher) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare the water bath to 56°C.*
- *Prepare 15 ml centrifuge tube.*
- *Prepare absolute ethanol in the freezer.*
- *All centrifugation should be performed at room temperature.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C.*

1. **Add 100 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 15 ml centrifuge tube (not provided).**

2. **Add 2 ml of sample to the tube, and vortex to mix thoroughly.**
   If the sample volume is less than 2 ml, bring the volume of sample to 2 ml with 1X PBS.

3. **Add 2.4 ml of Buffer JBL to the tube. Vortex the tube for 30 sec to mix thoroughly.**

4. **Incubate the tube for 20 min at 56°C.**
   During incubation with vortexing (every 5 min) helps accelerate lysis.

5. **Add 2.4 ml of chilled absolute ethanol (not provided) to the sample, and vortex 30 sec to mix the sample thoroughly.**

6. **Transfer 4 ml of the mixture to a column carefully. Close the cap, centrifuge for 3 min (4,500 rpm, RT).**

7. **Discard the pass-through and re-insert the column back into the 15 ml tube. Apply the remainder of the mixture, close the cap, and centrifuge for 3 min (4,500 rpm, RT).**

8. **Discard the pass-through and re-insert the column back into the 15 ml tube. Apply 2 ml of Buffer JBW and centrifuge for 3 min (4,500 rpm, RT).**

9. **Repeat step 8.**

10. **Discard the pass-through and re-insert the column back into the 15 ml tube. Apply 3 ml of Buffer NW, and centrifuge for 15 min (5,000 rpm, RT).** If the centrifugal force less than 4,500 x g. Centrifuge at full speed and discard the pass-through and re-insert the column back into the 15 ml tube. Centrifuge for 15 min at full speed.

11. **Place the column into a new 15 ml centrifugation tube (provided). Add 400 μl of Nuclease-free water to the center of membrane and close the cap. Incubate for 5 min at RT.**

12. **Centrifuge for 5 min (5,000 rpm, RT).**

13. ***(Optional :)*** **For higher yield, re-load the eluate from step 12 into the column, incubate 5 min at RT. Centrifuge for 5 min (5,000 rpm, RT).**

I.

PROTOCOL FOR
10 ml of Whole Blood (maxi)

Before experiment

- *Before first use, add absolute ethanol (ACS grade or higher) into Buffer JBW and NW as indicated on the bottle.*
- *Prepare the water bath to 65°C.*
- *Prepare 15 ml centrifuge tube.*
- *Prepare absolute ethanol in the freezer.*
- *All centrifugation should be performed at room temperature.*
- *If a precipitate has formed in Buffer JBL and JBW, heat to dissolve at 56°C.*

1. **Add 400 µl of Proteinase K solution (20 mg/ml, provided) into the bottom of a 50 ml centrifuge tube (not provided).**

2. **Add 10 ml of sample to the tube, and vortex to mix thoroughly.**
   If the sample volume is less than 10 ml, bring the volume of sample to 10 ml with 1X PBS.

3. **Add 12 ml of Buffer JBL to the tube. Vortex the tube for 30 sec to mix thoroughly.**

4. **Incubate the tube for 20 min at 65°C.**
   During incubation with vortexing (every 5 min) helps accelerate lysis.

5. **Add 12 ml of chilled absolute ethanol (not provided) to the sample, and vortex 30 sec to mix the sample thoroughly.**

6. **Transfer 17 ml of the mixture to a column carefully. Close the cap, centrifuge for 3 min at 4,500 rpm.**

7. **Discard the pass-through and re-insert the column back into the 50 ml tube. Apply the remainder of the mixture, close the cap, and centrifuge for 3 min (4,500 rpm, RT).**

8. **Discard the pass-through and re-insert the column back into the 50 ml tube. Apply 5 ml of Buffer JBW and centrifuge for 3 min (4,500 rpm, RT).**

9. **Repeat step 8.**

10. **Discard the pass-through and re-insert the column back into the 50 ml tube. Apply 10 ml of Buffer NW, and centrifuge for 15 min (5,000 rpm, RT).**
    If the centrifugal force less than 4,500 x g. Centrifuge at full speed and discard the pass-through and re-insert the column back into the 50 ml tube. Centrifuge for 15 min at full speed.

11. **Place the column into a new 50 ml centrifugation tube (provided). Add 1 ml of Nuclease-free water to the center of membrane and close the cap. Incubate for 5 min at RT.**

12. **Centrifuge for 5 min (5,000 rpm, RT).**

13. ***(Optional :)* For higher yield, re-load the eluate from step 12 into the column, incubate 5 min at RT. Centrifuge for 5 min (5,000 rpm, RT).**

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no yield	**Starting material is too aged or has been improperly stored**	The best yield is typically obtained from fresh samples. DNA yield depends on various factors, including the type, size, age, and storage condition of the starting material. Inappropriate storage, such as blood samples stored at 4°C for more than 5 days, may lead to reduced yields.
	Inefficient or insufficient lysis	For proper lysis, mix sample and lysis buffer thoroughly.
	Decreased proteinase K activity due to improper storage or expiration	Proteinase K must be stored at under 4°C to maintain proper activity after being dissolved in PK-Storage buffer. Proper lysis cannot be performed with degraded proteinase K. It should be replaced with a new one.
	Precipitation of Buffer JBL and JBW	Storage at cool ambient temperature may cause precipitation in Buffer JBL. Incubate bottle at 56°C or above until all precipitates are dissolved.
Low concentration of DNA in eluate	**Low sample input or a small number of cells in the sample**	Either add more starting material or, if needed, minimize the elution volume and re-elute the initial eluate.
	Column clogging	Insufficient lysis may lead to column clogging. Mix the sample with each buffer completely. Reduce the amount of starting sample. Extend the proteinase K incubation time at 56°C or double the amount of proteinase K.

Facts	Possible Causes	Suggestions
Eluate does not perform well In downstream Application	**PCR inhibitors present in samples**	PCR inhibitor in gDNA can obstruct PCR reaction. Dilute the elute to use template for PCR reactions.
	Incomplete removal of hemoglobin	In case of DNA preparation from certain animal blood, it is hard to remove hemoglobin from their blood. Carry out additional wash step with Buffer JBW before washing with Buffer NW.
	Buffer JBW or NW was prepared Incorrectly	Check that the Buffer JBW and NW concentrates were diluted with the correct volume of absolute ethanol. Repeat the extraction procedure with new samples, if available.
	Residual ethanol from Buffer JBW or NW that remains in the elute	Care must be taken for eliminating the carry-over of Buffer JBW or NW before elution step. The membrane of mini spin column should be kept completely dry using additional centrifugation or air-drying.
Low A_{260}/A_{280} ratio	**Insufficient lysis**	Insufficient lysis causes low DNA purity, and it is usually due to too much starting material used, imperfect mixing with Buffer JBL, insufficient time to lyse completely, or poor disruption of sample. Check these out in next preparations.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
	midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™* *for preparation of transfection-grade plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	midi	26	104-226	spin / vacuum
		100	104-201	
	maxi	10	104-310	spin / vacuum
		26	104-326	
Tissue Plus SV	mini	100	109-101	spin / vacuum
		250	109-152	
	midi	26	109-226	spin / vacuum
		100	109-201	
	maxi	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	midi	26	105-226	spin / vacuum
		100	105-201	
	maxi	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	maxi	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	midi	26	108-226	spin / vacuum
		100	108-201	
	maxi	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	midi	26	117-226	spin / vacuum
		100	117-201	
	maxi	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
Stool DNA mini	mini	50	115-150	spin
Stool-Bead DNA mini	mini	50	115-151	spin
Viral DNA/RNA	mini	50	128-150	spin
FFPE Tissue DNA	mini	50	138-150	spin
		250	138-152	

GeneAll® *GenEx™* *for isolation of total DNA without spin column*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *GenEx™* *for isolation of total DNA without spin column*				
GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant Plus	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	
GeneAll® *DirEx™* series *for preperation of PCR-template without extraction*				
DirEx™		100	250-101	solution
DirEx™ *Fast*-Tissue		96 T	260-011	solution
DirEx™ *Fast*-Cultured cell		96 T	260-021	solution
DirEx™ *Fast*-Whole blood		96 T	260-031	solution
DirEx™ *Fast*-Blood stain		96 T	260-041	solution
DirEx™ *Fast*-Hair		96 T	260-051	solution
DirEx™ *Fast*-Buccal swab		96 T	260-061	solution
DirEx™ *Fast*-Cigarette		96 T	260-071	solution
GeneAll® RNA series *for preperation of total RNA*				
RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ Plus	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD Plus	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed/Fruit	mini	50	317-150	spin
Ribospin™ Pathogen/TNA	mini	50	314-150	spin
		250	314-152	
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351- 001	solution

Products	Scale	Size	Cat. No.	Type
GeneAll® *AmpONE™* *for PCR amplification*				
Taq DNA polymerase		250 U	501-025	(2.5 U/μl)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix	20 μl x 96 tubes		526-200	solution
	50 μl x 96 tubes		526-500	
GeneAll® *AmpMaster™* *for PCR amplification*				
Taq Master mix	0.5 ml x 2 tubes		541-010	solution
	0.5 ml x 10 tubes		541-050	solution
GeneAll® *HyperScript™* *for Reverse Transcription*				
Reverse Transcriptase		10,000 U	601-100	solution
RT Master mix	0.5 ml x 2 tubes		601-710	solution
One-step RT-PCR Master mix	0.5 ml x 2 tubes		602-110	solution
One-step RT-PCR Premix	20 μl x 96 tubes		602-102	solution
GeneAll® RealAmp™ *for qPCR amplification*				
SYBR qPCR Master mix (2X, Low ROX)	200 rxn	2 ml	801-020	solution
	500 rxn	5 ml	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	2 ml	801-021	solution
	500 rxn	5 ml	801-051	
GeneAll® Protein series				
ProtinEx™ Animal cell/tissue		100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml x 10 tubes		751-001	solution

Products	Size	Cat. No.	Type
GeneAll® GENTi™ 32 ADVANCED *Newly designed automated extraction system*			
Automatic extraction equipment		GTI032A	system
Genomic DNA	48	901-048A	tube
	96	901-096A	plate
Viral DNA/RNA	48	902-048A	tube
	96	902-096A	plate
Blood DNA	48	903-048A	tube
	96	903-096A	plate
Plant DNA/RNA	48	904-048A	tube
	96	904-096A	plate
LMO	48	906-048A	tube
	96	906-096A	plate
Fecal DNA/RNA	48	913-048A	tube
	96	913-096A	plate
GeneAll® AllEx®64 *Compact yet Comprehensive automated extraction system*			
Automatic extraction equipment		AEX064	system
Genomic DNA	48	931-048A	tube
	96	931-096A	plate
Viral DNA/RNA	48	934-048A	tube
	96	934-096A	plate
Blood DNA	48	935-048A	tube
	96	935-096A	plate
Plant DNA/RNA	48	937-048A	tube
	96	937-096A	plate
Fecal DNA/RNA	48	948-048A	tube
	96	948-096A	plate

memo

memo

GeneAll

GENEALL BIOTECHNOLOGY CO., LTD
GeneAll Bldg., 303-7, Dongnamro, Songpa-gu, Seoul, Republic of Korea 05729
E-mail : sales@geneall.com
Tel. 82-2-407-0096 Fax. 82-2-407-0779
www.geneall.com

Manufacturer site

A-1201~A-1204, Hanam Techno Valley U1 Center,
947, Hanam-daero, Hanam-si, Gyeonggi-do, 12982, Republic of Korea

2024.03