Ver 3.2

Handbook for

- TISSUE SV MINI
  TISSUE SV MIDI
  TISSUE SV MAXI

exgene tm

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.

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This protocol handbook is included in :

GeneAll® Exgene™ Tissue SV mini (104-101, 104-152)
GeneAll® Exgene™ Tissue SV Midi (104-226, 104-201)
GeneAll® Exgene™ Tissue SV MAXI (104-310, 104-326)
GeneAll® Exgene™ Tissue SV *Plus!* mini (109-101, 109-152)
GeneAll® Exgene™ Tissue SV *Plus!* Midi (109-226, 109-201)
GeneAll® Exgene™ Tissue SV *Plus!* MAXI (109-310, 109-326)

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KIT CONTENTS

GeneAll® Exgene™ Tissue SV (*plus!*) Series

Cat. No.	104(9)-101	104(9)-152	104(9)-226
Size	**mini**	**mini**	**Midi**
No. of preparation	100	250	26
Column Type G	100	250	26
2 ml Collection tube	300	750	52
Buffer RL	(100 ml)	(250 ml)	(200 ml)
Buffer TL	30 ml	80 ml	30 ml
Buffer TB	50 ml	110 ml	60 ml
Buffer BW (concentrate)	40 ml	90 ml	40 ml
Buffer TW (concentrate) ***	24 ml	50 ml	24 ml
Buffer AE *	30 ml	60 ml	30 ml
Proteinase K **	48 mg	120 mg	60 mg
PK Storage Buffer **	4 ml	7 ml	4 ml
Protocol Handbook	1	1	1

Cat. No.	104(9)-201	104(9)-310	104(9)-326
Size	**Midi**	**MAXI**	**MAXI**
No. of preparation	100	10	26
Column Type G	100	10	26
2 ml Collection tube	200	20	52
Buffer RL	(250 ml x 3)	(200 ml)	(250 ml x 2)
Buffer TL	120 ml	40 ml	100 ml
Buffer TB	240 ml	80 ml	240 ml
Buffer BW (concentrate)	90 ml x 2	40 ml	90 ml
Buffer TW (concentrate) ***	50 ml x 2	24 ml	50 ml x 2
Buffer AE *	120 ml	30 ml	60 ml
Proteinase K **	240 mg	48 mg	120 mg
PK Storage Buffer **	14 ml	4 ml	7 ml
Protocol Handbook	1	1	1

** Before first use, add absolute ethanol (ACS grade or better) into Buffer BW and TW as indicated on the bottle.*

† Contains sodium azide as a preservative.

*** 10 mM TrisCl, pH 9.0, 0.5 mM EDTA.*

**** For the long-term storage of Proteinase K, store at 4 °C. But after reconstitution of Proteinase K store at -20 °C.*
Refer to instruction of Proteinase K on page 7.

INTENDED USE

GeneAll® Exgene™ Tissue SV kit provides the components and procedures necessary for purifying total DNA from animal tissues, cultured cells, gram negative bacteria, insects and FFPE tissues.

Exgene™ Tissue SV *plus!* kit additionally provides the method for purifying total DNA from mammalian whole blood and its derivatives. For other procedures and additional information, contact our technical support team.

STORAGE CONDITION

All components of GeneAll® Exgene™ Tissue SV kit should be stored at room temperature. After reconstitution of Proteinase K with storage buffer, it should be stored under 4°C for conservation of activity. It can be stored at 4°C for 1 year without significant decrease in activity. But for prolonged preservation of activity, storage under -20°C is recommended.

During shipment or storage under cold ambient condition, a precipitate may be formed in Buffer TB. In such a case, heat the bottle at 37°C to dissolve completely. It will bring about poor result to use the precipitated buffer as it is.

Exgene™ Tissue SV kits are guaranteed for 18 months from the production date.

QUALITY CONTROL

All components in GeneAll® Exgene™ Tissue SV kit are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically.

Restriction enzyme assay, PCR amplification assay and spectrophotometric assay as quality control are carried out from lot to lot thoroughly, and only the qualified is approved to be delivered.

SAFETY INFORMATION

The buffers included in GeneAll® Exgene™ Tissue SV Kit contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

Buffer TB contains chaotropes. It can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample-preparation waste. Please refer to Material Safety Data Sheet (MSDS) for more detailed informations.

PRODUCT SPECIFICATIONS

Exgene™ Tissue SV			
	mini	**Midi ***	**MAXI ***
Maximum sample size	~25 mg	~100 mg	~250 mg
Preparation time	25 min~ **	40 min~ **	40 min~ **
Maximum loading volume	750 μl	5 ml	16 ml
Binding capacity	60 μg	200 μg	600 μg
Elution volume	30-400 μl	200-600 μl	0.4-2 ml

** Exgene™ Tissue SV Midi/MAXI Kit procedures require the centrifuge which has a swining-bucket rotor and ability of 4,000~5,000 x g.*

*** Depends on the complete lysis time of sample materials.*

PREPARATION OF PROTEINASE K SOLUTION

Proteinase K in Exgene™ series is provided in a freeze-dried format. Thus, it should be reconstituted thoroughly with PK storage buffer before experiment. PK storage buffer contains calcium chloride and glycerol which do not affect the enzyme activity, but contribute to its stability. Please reconstitute Proteinase K solution as printed on its label. Reconstituted enzyme should be stored at 4°C for its stability.

INTRODUCTION

GeneAll® Exgene™ Tissue SV series are designed for puryfing total DNA from various animal tissue samples including animal tissues, cultured cells, gram negative bacteria, insects, FFPE tissues and whole blood. Purified DNA can be used for PCR, blotting, enzyme assays and many downstream applications which require high purity of nucleic acids.

Exgene™ series utilize the advanced silica-binding technology to purify total DNA sufficiently pure for many applications. Various samples are lysed in optimized buffers containing detergents and lytic enzyme to rip off the cellular membrane. Under high salt condition, the nucleic acid in the lysate bind specifically to the surface of silica membrane. The impurities bound nonspecifically are washed away with a series of washing buffers, and finally pure DNA is released into a clean collection tube by deionized water or low ionic strength buffer.

SAMPLE AMOUNT AND EXPECTED YIELD

The amount of starting sample should not be exceed the maximum limit, otherwise DNA recover will be significantly lowered. (Fig. 1) Recommended amount of starting sample and the yield is listed on next page.

If your starting material is not listed or you have no information about your sample, we recommend you start with smaller sample than the listed and increase the sample size in subsequent preparation depending on the result.

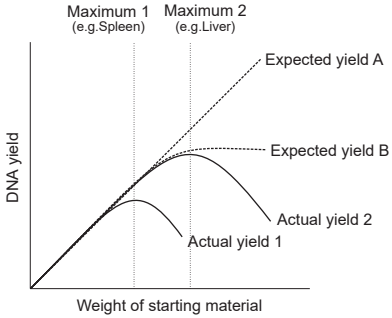

Fig. 1 Starting sample amount should not be exceed the maximum limit, otherwise DNA recover will be significantly lowered. If the cell mass of starting material is high (e.g., spleen, actual yield 1), maximum capacity will be lowered. (Maximum 1)

DISRUPTION AND LYSIS

Generally, to make the sample finer will result in not only better yield and quality of DNA, but also reduced lysis time. Grinding in mortar and pestle under liquid nitrogen is a good method for disrupting the tissue sample, but alternative methods, such as a homogenizer or a bead-beater, can be employed in case by case. Shaking or vortexing during incubation for lysis may greatly accelerate the efficiency of lysis, followed by reduced time for complete lysis. Note that the freshness and the particle size of ground sample is the key for good result and that the fresh or frozen sample should be kept on ice until use.

Table 1. Maximum amount of starting sample

Sample	Maximum amount		
	mini	Midi	MAXI
Animal tissue	25 mg	100 mg	250 mg
Mammalian blood *	300 μl	2 ml	5 ml
Mouse tail	1 cm	-	-
Rat tail	0.6 cm	-	-
Insect	50 mg	200 mg	500 mg
Cultured cell	5×10^6	2×10^7	5×10^7
Bacteria	2×10^9	8×10^9	2×10^{10}

Table 2. Typical DNA yields from various sample using Exgene™ Tissue SV mini

The yield of this table is calculated by addition of each eluate of 3 successive elution steps after DNA preparation with RNase A treatment. Without RNase A treatment, average yield from some sample may be significantly different from these data.

Sample	Starting amount	Yield (μg)
Brain	20 mg	5-18
Heart	20 mg	4-10
Kidney	15 mg	15-35
Liver	20 mg	15-35
Lung	20 mg	4-10
Pancreas	20 mg	8-25
Spleen	10 mg	10-35
Rat tail	0.6 cm	15-35
Cultured cell	2×10^6	10-25
Lymphocytes	5×10^6	10-25
Whole blood	300 μl	5-15
Bacteria	2×10^9	5-25

ELUTION

Purified DNA can be eluted from SV column membrane in either deionized water or Buffer AE which contains 0.5 mM EDTA and 10 mM Tris-HCl, pH 9.0. Elution buffer should be equilibrated to room temperature before applying to SV column. Typically in mini kit, elution is carried out in two successive steps using 200 μl Buffer AE each time. The volume of elution can be adjusted depending on the starting materials or the downstream applications, but it should be over the minimum requirements to wet the entire column membrane (50 μl per column for mini) and should not be over 300 μl. Basically, it is recommended for recovery of higher DNA concentration to decrease the elution volume to minimum, but total DNA recovery will decrease in this case. Otherwise, if maximum recovery is needed, the volume of elution buffer should be increased to elute as much as possible. But it can not be over 300 μl which is a physical limit.

Unless the starting material has very low cell density, DNA bound to the SV column membrane may not be eluted completely with a single elution step. Approximately 60~85% of DNA will be eluted in the first eluate (Fig. 2) However, a single elution with recommended volume of elution buffer will be sufficient to recover the amount of DNA required for multiple PCR reactions.

For very small samples (containing less than 1 μg of DNA), only a single elution in 50 μl of Buffer AE or deionized water will be sufficient for complete elution of DNA.

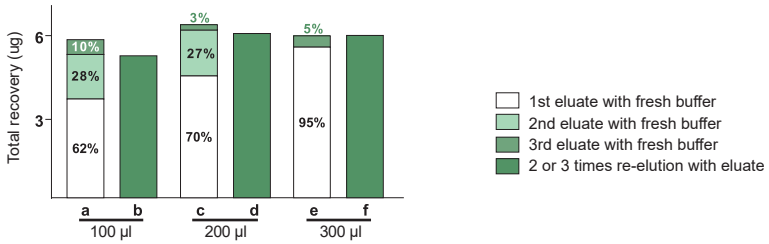

Fig. 2 The yield and concentration of purified DNA depending on the elution procedures.

Total DNA was prepared from 10 mg of mouse liver using Exgene™ Tissue SV mini. Each preparation was exactly identical except the elution procedure; Elution was performed 3 times per column with 100 μl (a) and 200 μl (c), or 2 times per column with 300 μl (c) of fresh Buffer AE. At the same time, another elution was carried out 3 times (b, d) or 2 times (f) by recursive use of eluate instead of fresh Buffer AE.

CENTRIFUGE IN MIDI/MAXI KITS

Exgene™ SV 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 inconsistent contact of SV column membrane with mixtures and/or buffers. Low g-force will lead to uncomplete removal of ethanol from SV column membrane and reduced volume of eluate. Usable centrifuges and rotors were listed below, but you can employ any equivalent.

Company	Centrifuge	Rotor
Beckman Coulter Inc. (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

GeneAll®

Tissue and Tissue *plus!* SV mini

Read the protocol carefully before experiment.

A. PROTOCOL FOR ANIMAL TISSUE

Before experiment

- Prepare the water bath to 56°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, completely dissolve it in 37°C water bath.

1. Disrupt 20 mg of tissue as described on step 1a, 1b or 1c, depending on the sample type.

To disrupt the sample finer will accelerate lysis and reduce the lysis time.
For spleen tissue, up to 10 mg can be processed.
If the starting sample is larger than 25 mg (if spleen, 10 mg), increase the volume of Buffer TL proportionally. For 50 mg of liver tissue, 400 µl of Buffer TL is required.
For over 50 mg of tissue, Exgene™ Tissue SV Midi or MAXI is recommended.

1a. For soft tissue, such as liver or brain, put 20 mg of the tissue into 1.5 ml tube, add 200 µl of Buffer TL, and homogenize thoroughly with microhomogenizer.

1b. If microhomogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put 20 mg of the powdered tissue into 1.5 ml tube. Add 200 µl of Buffer TL and pulse-vortex for 15 sec.

1c. If neither 1a nor 1b is available, mince 20 mg of tissue with sharp blade or scalpel as small as possible. Put the tissue into a 1.5 ml tube. Add 200 µl of Buffer TL and pulse-vortex for 15 sec.

***** Alternatively, tissue samples can be effectively disrupted using some instruments, such as a rotor-stator homogenizer or a bead-beater.**

2. **Add 20 μl of Proteinase K solution. Mix completely by vortexing or pipetting. Incubate at 56°C until the sample is completely lysed.**
   It is essential to mix the components completely for proper lysis.
   If the sample amount is larger than 25 mg (if spleen, 10 mg), increase the amount of Proteinase K proportionally. For 50 mg of liver tissue, 40 μl of Proteinase K solution is required.
   Lysis time varies from 10 min to 3 hr usually depending on the type of tissue processed and the disruption method (step 1). The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.
   If the sample tube is incubated in water bath or heating block, vortex occasionally (2-3 times per hour) during incubation to lyse readily. *Lysis in shaking water bath, shaking incubator or agitator would be best for efficient lysis.*

3. **Check!! If Buffer TB precipitates, pre-heat in a 37°C water bath to dissolve completely.**
   Precipitated buffer will cause significant decrease in recover yield.

4. **Spin down the tube briefly to remove any drops from inside of the lid.**

5. ***Optional:* If RNA-free DNA is required, add 4 μl of RNase solution (100 mg/ml, Cat. No. 117-960), vortex to mix thoroughly, and incubate for 2 min at room temperature.**
   Exgene™ Tissue SV column has stronger affinity to DNA than RNA. Although the treatment of RNase is omitted, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

6. **Add 400 μl of Buffer TB. Immediately vortex the tube to mix thoroughly. Spin down the tube briefly to remove any drops from inside of the lid.**
   If the sample is larger than 25 mg (if spleen, 10 mg), increase the volume of Buffer TB proportionally. For 50 mg of liver tissue, 800 μl of Buffer TB is required.

7. **Apply the mixture to the SV column. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one (provided).**
If more than 25 mg (10 mg spleen) of tissue is processed, apply the mixture twice; apply 700 µl of the mixture, spin down, discard the filtrate, re-insert to the empty collection tube, and repeat the step again until all of the mixture is applied to the SV column. If the mixture has not passed completely through the membrane, centrifuge again at full speed until all of the solution has been passed through.
Centrifuge at maximum speed will not affect the DNA recovery.

8. **Add 600 µl of Buffer BW. Centrifuge for 30 sec at 6,000 x g above (>8,000 rpm). Replace the collection tube with new one (provided).**

9. **Apply 700 µl of Buffer TW. Centrifuge for 30 sec at 6,000 x g above (>8,000 rpm). Discard the filtrate and reinsert the SV column back into the collection tube.**

10. **Centrifuge at full speed (above 13,000 x g) for 1 min to remove residual wash buffer. Place the SV column in a fresh 1.5 ml tube (not provided).**
Care must be taken at this step for eliminating the carryover of Buffer TW. If carryover of Buffer TW occurs on column membrane, centrifuge again for 1 min at full speed before transferring to the new 1.5 ml tube.

11. Add 200 μl of Buffer AE or sterilized water. Incubate for 2 min at room temperature. Centrifuge at full speed (>13,000 x g) for 1 min.

Ensure that the Buffer AE or sterilized water is dispensed directly onto the center of SV column membrane for optimal elution of DNA.

Normally repeat of elution step with fresh 200 μl elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 μl of elution buffer will increase yields slightly. Each eluate can be separated in fresh tubes or can be collected to same tube, but more than 300 μl of eluate can not be collected in a 1.5 ml tube because the SV column will come into contact with the eluate.

If higher concentration of DNA is needed or starting sample amount is very small, second elution can be carried out with the first eluate instead of the fresh elution buffer. Alternatively or simultaneously, elution volume can be decreased to 50 μl for higher DNA concentration. However the small volume of elution buffer will decrease the total yield of DNA recovery.

For long-term storage, eluting in Buffer AE is recommended. But EDTA included in Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled deionized water (>pH 7.0) or Tris-Cl (>pH 8.5). *When using water for elution, check the pH (>7.0) of water before elution.*

B. PROTOCOL FOR RODENT TAIL

Before experiment

- Prepare the water bath to 56°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, dissolve it in 37°C water bath.
- Prepare Ice and 0.5 M EDTA solution (pH 8.0).

1. **Add 30 µl of a 0.5 M EDTA solution (pH 8.0) to 180 µl of Buffer TL in a 1.5 ml centrifuge tube. Chill on ice before use.**

2. **Mince 0.3-1.0 cm of mouse or rat tail as small as possible. Transfer it to the tube containing chilled EDTA-Buffer TL mixture.**
   For rodent tail tip, up to 0.6 cm (rat or adult mouse) or 1.0 cm (mouse) can be processed for each prep.
   The tail should be submerged in EDTA-Buffer TL mixture.

*** ***Alternatively, tissue samples can be effectively disrupted using some instruments, such as a rotor-stator homogenizer or a bead-beater.***

3. **Add 20 µl of Proteinase K solution (20 mg/ml, provided).**

4. **Incubate at 56˚C until the tissue is completely lysed.**
   It can take up to 8 hours to lysis completely. Vortex occasionally during incubation, or incubate on shaking or agitating platform. *Make sure the tail is completely digested.*
   Overnight lysis does not affect DNA recovery.
   Complete lysate may appear clear and viscous. If the lysate appears to be gelatinous or has leftover particles, increase the lysis time or the volume of EDTA-Buffer TL and Proteinase K. Remember that the volume of Buffer TB should be increased proportionally in subsequent step.

5. **Continue with step 3 of Animal Tissue protocol (page 14).**

C. PROTOCOL FOR CULTURED ANIMAL CELL OR LYMPHOCYTES

Before experiment

- Prepare the water bath to 56°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, completely dissolve it in 37°C water bath.

* Washing cells with sufficient volume of PBS before procedures usually brings about better results.

1. **Harvest cells (up to 5 x 10^6 cells) to a 1.5 ml microcentrifuge tube by centrifugation at 14,000 x g for 20 sec.**
   Alternatively, cells can be pelleted at 1,000 x g for 5 min. For adherent cells, trypsinize the cells before harvesting.
   Certain cells, such as PC12, do not lyse well in Buffer TL. For those cells, it is helpful to perform additional freeze-thaw step several times before proceeding to next step.

2. **Discard the supernatant as much as possible and thoroughly resuspend cell pellet in 200 µl of Buffer TL.**
   For efficient resuspending in Buffer TL, it is helpful to loosen and resuspend the pellets by flickering or vortexing before the addition of Buffer TL.

3. **Add 20 µl of Proteinase K solution. Mix completely by vortexing, or pipetting. Incubate for 10 min at 56°C.**
   Longer incubation will not affect DNA recovery.

4. **Continue with Animal Tissue Protocols from step 3 (page 14).**

D. PROTOCOL FOR INSECTS OR WORMS

Before experiment

- Prepare the water bath to 56°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, completely dissolve it in 37°C water bath.

1. **Grind up to 50 mg (20 mg, if worms) of insect in liquid nitrogen with pre-chilled mortar and pestle.**
   Place the powder in a 1.5 ml microcentrifuge tube.
   Fine powder will reduce lysis time and bring about better result.
   Worms can be minced using sharp blade or scalpel instead of grinding in liquid nitrogen.

*** ***Alternatively, tissue samples can be effectively disrupted using some instruments, such as a rotor-stator homogenizer or a bead-beater.***

2. **Apply 200 µl of Buffer TL and mix completely by vortexing or pipetting.**

3. **Add 20 µl of Proteinase K solution. Mix completely by vortexing and incubate at 56˚C for 30 mins.**
   It is essential to mix the components completely for proper lysis.
   To mix occasionally during incubation will greatly accelarate the lysis and help reduce the lysis time.
   Lysis time can vary depending on the type and amount of starting sample. Samples can be further incubated for complete lysis and longer incubation will not affect the recover yield.
   After complete lysis, the lysate will turn to clear or transparent from turbid, but it may still have some remaining debris originated from the sample, such as exoskeleton.

4. ***Optional:*** **If some debris remain in the mixture after lysis, spin down briefly to pellet the debris, and transfer the supernatant to a fresh 1.5 ml tube.**

5. **Continue with Animal Tissue Protocols from step 3 at page 14.**

E. PROTOCOL FOR PARAFFIN-FIXED TISSUE

Before experiment

- Prepare xylene, absolute ethanol.
- Prepare the water bath to 56°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, completely dissolve it in 37°C water bath.

1. **Place a small section of paraffin-fixed tissue in a 2 ml microcentrifuge tube.**
   Start with smaller sample. If DNA yield is smaller than expected, increase the amount of sample gradually in next preparations.

2. **Add 1,200 µl xylene. Vortex vigorously.**

3. **Centrifuge at full speed (>13,000 x g) for 5 min. Carefully remove supernatant by pipetting.**
   Do not remove any of the pellet.

4. **Add 1,200 µl of absolute ethanol to the pellet to remove residual xylene and mix gently by vortexing.**

5. **Centrifuge at full speed for 5 min. Carefully remove the ethanol by pipetting.**
   Do not remove any of the pellet.

6. **Repeat steps 4-5 for 2 times or more.**

7. **Evaporate the ethanol by incubating the microcentrifuge tube at room temperature for 10-15 min with open cap.**

8. **Resuspend the tissue pellet in 200 µl Buffer TL and follow the tissue protocol from step 2 (page 14).**

F. PROTOCOL FOR GRAM NEGATIVE BACTERIA

Before experiment

- Prepare the water bath to 56°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, completely dissolve it in 37°C water bath.

1. **Pellet bacterial cells (up to 2 x10^9 cells) to a 1.5 ml microcentrifuge tube by centrifugation at 14,000 x g for 30 sec.**
   1~2 ml of overnight bacterial liquid culture (OD_{600}=1) may correspond to 1 x 10^9 cells.

2. **Discard the supernatant as much as possible and thoroughly resuspend bacterial pellet in 200 µl of Buffer TL.**
   For efficient resuspending in Buffer TL, it is helpful to loosen and resuspend the pellets by flickering or vortexing before the addition of Buffer TL.

3. **Add 20 µl of Proteinase K solution. Mix completely by vortexing or pipetting. Incubate for 10 min at 56˚C.**
   After complete lysis, lysis mixture will turn to clear from turbid. If the 10 min lysate still looks turbid or cloudy, incubate until the lysate become clear without any particle.
   Lysis time may vary depending on the species and cell numbers. Cells can be further incubated for complete lysis and longer incubation time does not affect recover yield. After incubation, cool the lysate to room temperature.

4. **Continue with Animal Tissue Protocols from step 3 (page 14).**

G. PROTOCOL FOR MAMMALIAN WHOLE BLOOD *(PLUS! ONLY)*

Before experiment

- *Prepare the water bath to 56°C.*
- *Equilibrate Buffer AE to room temperature.*
- *All centrifugation should be performed at room temperature.*
- *Buffer TB may precipitate at cold ambient temperature.*
- *If so, completely dissolve it in 37°C water bath.*

* For convenient preparation from whole blood, use Exgene™ Blood SV kit.

1. **Transfer 300 μl of whole blood to a new 1.5 ml tube.**
   Before transfer of blood, it is recommended that gently rocking the tube of blood until throughly mixed.

2. **Add 900 μl of Buffer RL to the tube containing the blood sample. Invert the tube 5-6 times to mix. Incubate the mixture for 10 min at room temperature.**
   Invert 2-3 times during the incubation. The lysate should become clear (translucent). If the lysate is turbid (opaque) not clear, it may be frozen or mis-stored sample, and you should read the annotation of step 3.
   If fresh or well-stored sample is processed, it will take less times than 10 min to acquire translucent mixtures.
   For larger blood sample, we recommend Exgene™ Blood SV Midi/MAXI kit. For simple and convenient preparation from blood samples, we recommend Exgene™ Blood SV mini (105-101, 105-152). This kit provides the rapid and easy method for the purification of total DNA from up to 400 μl of whole blood or its derivative in addition to lymphocytes and cultured cells.

3. **Centrifuge for 1 min at 14,000 x g (full speed) and carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet by vortexing or flickering.**
   Approximately 10-20 μl of residual liquid will remain. Steps 2-3 are critical steps for DNA recovery, so you have to check the translucent lysate and the white (or pink) pellet before processing next step. If blood sample has been frozen or mis-stored, resuspend the pellet and repeat step 2-3 with the resuspended cells until the lysate becomes translucent.

4. **Check!! If Buffer TB precipitates, pre-heat in a 37˚C water bath to dissolve completely.**

5. **Add 200 μl of Buffer TL to the tube containing the resuspended cells. Pipet the solution 5-6 times to mix well.**
   The lysate may be viscous.

6. **Add 10 μl of Proteinase K solution. Mix thoroughly by vortexing or pipetting. Incubate for 10 min at 56˚C.**
   Overnight lysis is available and it will not influence the preparation.

7. **Continue with Animal Tissue Protocols from step 4 (page 14).**

GeneAll®

Exgene™ Tissue SV Midi

Visit www.geneall.com for more Midi protocols.

A. PROTOCOL FOR ANIMAL TISSUE

Before experiment

- Prepare the water bath to 64°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, sompletely dissolve it in 37°C water bath.

Important Note!

All centrifugation steps MUST be carried out on a tabletop centrifuge which has an ability of 4,000 x g at least and a swing-out bucket.
DO NOT USE A FIXED-ANGLE ROTOR.

1. Disrupt 30~100 mg of tissue as described in step 1a, 1b or 1c, depending on the sample type.

Finer sample powder will accelerate lysis and decrease the lysis time.
For spleen tissue, up to 40 mg can be processed. For over 100 mg of tissue, Exgene™ Tissue SV MAXI (up to 250 mg) is recommended.

1a. For soft tissue, such as liver or brain, put up to 100 mg of the tissue into 15 ml conical tube, add 400 μl of Buffer TL, homogenize thoroughly with microhomogenizer, add 600 μl of Buffer TL, and vortex vigorously to resuspend well.

1b. If microhomogenizer is not available or the tissue is not soft, grind the tissue to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 100 mg of the powdered tissue into 15 ml conical tube. Add 1 ml of Buffer TL and pulse-vortex for 30 sec.

1c. If neither 1a nor 1b is available, mince the tissue with sharp blade or scalpel as small as possible. Put up to 100 mg of the tissue into a 15 ml conical tube. Add 1 ml of Buffer TL and pulse-vortex for 30 sec.

*** ***Alternatively, tissue samples can be effectively disrupted using some instruments, such as a rotor-stator homogenizer or a bead-beater.***

2. **Add 100 μl of Proteinase K solution to the tube. Mix completely by vortexing or pipetting. Incubate at 64˚C until the sample is completely lysed.**

   It is essential to mix the components completely for proper lysis.

   Lysis time varies from 10 min to 3 hr usually depending on the type of tissue processed and the disruption method (step 1). The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.

   If the sample tube is incubated in water bath or heating block, vortex occasionally (2-3 times per hour) during incubation to lysis readily. *Incubation in shaking water bath, shaking incubator, or agitator would be best for efficient sample lysis.*

3. **Check!! If Buffer TB precipitates, pre-heat in a 37˚C water bath to dissolve completely.**

   Precipitated buffer will cause significant decrease in recover yield.

4. ***Optional:* If RNA-free DNA is required, add 20 μl of RNase solution (100 mg/ml, Cat. No. 117-960), vortex to mix thoroughly, and incubate for 3 min at room temperature.**

   Exgene™ Tissue SV column has stronger affinity to DNA than RNA. Although the treatment of RNase is omitted, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

5. **Add 2 ml of Buffer TB. Immediately vortex or invert the tube to mix thoroughly.**

   Check that Buffer TB is not precipitated. For efficient binding of DNA to membrane, it is essential to mix the sample thoroughly to yield a homogeneous solution.

6. **Transfer the sample mixture to the SV Midi column carefully, close the cap, centrifuge for 2 min at 3,000 x g (3,800 rpm).**

   There may be appear some floating matters in mixture. It does not affect DNA recovery, so it is recommended transfer of even that matters.

   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the SV column back into the 15 ml tube. Apply 3 ml of Buffer BW and centrifuge for 2 min at 3,000 x g (3,800 rpm).**
   While transfer of mixture to the SV column, be careful not to moisten the rim of SV column.

8. **Discard the filtrate, wipe off any spillage from the thread of the 15 ml tube, and re-insert the SV column back into the 15 ml tube.**

9. **Carefully, without moistening of the rim, apply 4 ml of Buffer TW, and centrifuge for 15 min at 4,500 x g (5,000 rpm).**
   At least, 4,000 x g is required for proper DNA recovery.
   Insufficient centrifugal force may lead to remaining of ethanol in SV column membrane, followed by poor DNA recovery.
   If the SV column has Buffer TW associated with it after centrifugation, incubate the SV column for 10~15 min at room temperature to evaporate residual ethanol. Residual ethanol may inhibit some downstream reactions, such as PCR.

10. **Place the SV column into a new 15 ml centrifugation tube (provided). Pipet 300 μl of Buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 2 min at room temperature. Centrifuge at full speed (over 4,000 x g, at least) for 5 min.**

    Lower centrifugal force will dramatically reduce the volume of eluate.

    Before this elution step, it is strongly recommended that any residual ethanol originated from Buffer TW should not remain in SV column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.

    Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the SV column membrane for optimal elution of DNA.

    For blood sample, if starting volume is less than 1 ml, apply 200 μl of Buffer AE or distilled water. Do not reduce the elution volume below 100 μl.

    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in the Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH 7.0) or Tris-Cl (>pH 8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

11. **For higher concentrated yield, re-load the eluate from step 11 into the SV column, close the cap, incubate 2 min at room temperature, and centrifuge at full speed for 5 min.**

    For higher total yield, add 300 μl of fresh Buffer AE or distilled water again into the SV column, close the cap, incubate 2 min at room temperature, and centrifuge at full speed for 5 min.

    Less than 300 μl of eluate will be obtained from 300 μl of elution buffer, but this has no influence on DNA yields.

B. PROTOCOL FOR CELLS

Before experiment

- Prepare the water bath to 64°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, sompletely dissolve it in 37°C water bath.

Important Note!

All centrifugation steps MUST be carried out on a tabletop centrifuge which has an ability of 4,000 x g at least and a swing-out bucket.
DO NOT USE A FIXED-ANGLE ROTOR.

1. **Harvest cells listed below to a 15 ml conical tube by centrifugation at 2,000 x g for 10 min.**
   Cultured cells or lymphocytes : up to 2 x 10^7
   Bacterial cells : up to 8 x 10^9

2. **Discard the supernatant as much as possible and thoroughly resuspend cell pellet in 1 ml of Buffer TL.**
   For efficient resuspending in Buffer TL, it is helpful to loosen and resuspend the pellets by flickering or vortexing before the addition of Buffer TL.

3. **Add 100 µl of Proteinase K solution. Mix completely by vortexing or pipetting. Incubate for 15 min at 64°C.**
   Longer incubation will not affect DNA recovery.

4. **Continue with Animal Tissue Protocols from step 3 (page 27).**

C. PROTOCOL FOR MAMMALIAN WHOLE BLOOD *(PLUS! ONLY)*

* For convenient preparation from whole blood, use Exgene™ Blood SV kit.

Before experiment

- *Prepare the water bath to 64°C.*
- *Equilibrate Buffer AE to room temperature.*
- *All centrifugation should be performed at room temperature.*
- *Buffer TB may precipitate at cold ambient temperature.*
- *If so, sompletely dissolve it in 37°C water bath.*

Important Note!

All centrifugation steps MUST be carried out on a tabletop centrifuge which has an ability of 4,000 x g at least and a swing-out bucket.
DO NOT USE A FIXED-ANGLE ROTOR.

1. **Transfer 2 ml of whole blood to a new 15 ml conical tube.**
   Before transfer of blood, it is recommended that gently rocking the tube of blood until throughly mixed.

2. **Add 6 ml of Buffer RL to the tube containing the blood sample. Invert the tube 5-6 times to mix. Incubate the mixture for 10 min at room temperature.**
   Invert 2-3 times during the incubation. The lysate should become clear (translucent). If the lysate is turbid (opaque) not clear, it may be frozen or mis-stored sample, and you should read the annotation of step 3.
   For simple and convenient preparation from blood samples, we recommend Exgene™ Blood SV.
   For larger blood sample, we recommend Exgene™ Tissue SV *plus!* MAXI.

3. **Centrifuge for 10 min at 2,000 x g and carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet by vortexing or flickering.**
   A little of residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white (or pink) pellet before processing next steps.
   If blood sample has been frozen or mis-stored, resuspend the pellet and repeat step 2-3 with the sample until the lysate becomes translucent.

4. **Add 1 ml of Buffer TL to the tube containing the resuspended cells. Pipet the solution 5-6 times to mix well.**
   The lysate may be viscous.

5. **Add 60 µl of Proteinase K solution. Mix thoroughly by vortexing. Incubate for 15 min at 64˚C.**

6. **Check!! If Buffer TB precipitates, pre-heat in a 37˚C water bath to dissolve completely.**
   Precipitated buffer will cause significant decrease in recover yield.

7. **Continue with Animal Tissue Protocols from step 4 (page 27).**

GeneAll®

Exgene™ Tissue SV MAXI

Visit www.geneall.com for more MAXI protocols.

A. PROTOCOL FOR ANIMAL TISSUE

Before experiment

- Prepare the water bath to 64°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, sompletely dissolve it in 37°C water bath.

Important Note!

All centrifugation steps MUST be carried out on a tabletop centrifuge which has an ability of 4,000 x g at least and a swing-out bucket.
DO NOT USE A FIXED-ANGLE ROTOR.

1. Disrupt 100~250 mg of tissue as described in step 1a, 1b or 1c, depending on the sample type.

To make the sample finer will accelerate lysis and decrease the lysis time.

For spleen tissue, up to 100 mg can be processed.

1a. Disrupt tissue sample using a hand-held homogenizer or a rotor-stator homogenizer in 3 ml of Buffer TL. Be careful not to overflow due to foaming.

1b. If any homogenizer is not available, grind tissue sample to a fine powder with liquid nitrogen in a pre-chilled mortar and pestle. Put up to 250 mg of the powdered tissue into 15 ml conical tube. Add 3 ml of Buffer TL and pulse-vortex for 30 sec.

1c. If neither 1a nor 1b is available, mince the tissue with sharp blade or scalpel as small as possible. Put up to 250 mg of the tissue into a 15 ml conical tube. Add 3 ml of Buffer TL and pulse-vortex for 30 sec.

*** ***Alternatively, tissue samples can be effectively disrupted using some instruments, such as a bead-beater.***

2. **Add 200 μl of Proteinase K solution to the tube. Mix completely by vortexing or pipetting. Incubate at 64°C until the sample is completely lysed.**

   It is essential to mix the components completely for proper lysis.
   Lysis time varies from 10 min to 3 hr depending on the type of tissue processed and the disruption method (step 1). The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.
   If the sample tube is incubated in water bath or heating block, vortex occasionally (3-4 times per hour) during incubation to lysis readily. *Incubation in shaking water bath, shaking incubator, or agitator would be best for efficient sample lysis.*

3. **Check!! If Buffer TB precipitates, pre-heat in a 37°C water bath to dissolve completely.**

   Precipitated buffer will cause significant decrease in recover yield.

4. ***Optional*: If RNA-free DNA is required, add 200 μl of RNase solution (100 mg/ml, Cat. No. 117-961), vortex to mix thoroughly, and incubate for 3 min at room temperature.**

   Exgene™ Tissue SV column has the very stronger affinity to DNA than RNA. Although the treatment of RNase is omitted, RNA occupies very small portion of eluates. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR.

5. **Add 7 ml of Buffer TB. Immediately vortex or invert the tube to mix thoroughly.**

   Check that Buffer TB is not precipitated.
   For efficient binding of DNA to membrane, it is essential to mix the sample thoroughly to yield a homogeneous solution.

6. **Transfer the sample mixture to the SV MAXI column carefully, close the cap, centrifuge for 2 min at 2,000 x g (3,000 rpm).**

   There may be appear some floating matters in mixture. It does not affect DNA recovery, so it is recommended to transfer even that matters.
   If the mixture has not passed completely through the membrane, centrifuge again at higher speed until all of the solution has passed through.

7. **Discard the filtrate and re-insert the SV column back into the 50 ml tube. Apply 7 ml of Buffer BW and centrifuge for 2 min at 2,000 x g (3,000 rpm).**
   While transfer of mixture to the SV column, be careful not to moisten the rim of SV column.

8. **Discard the filtrate, wipe off any spillage from the thread of the 50 ml tube, and re-insert the SV column back into the 50 ml tube.**

9. **Carefully, without moistening of the rim, apply 10 ml of Buffer TW, and centrifuge for 15 min at 4,500 x g (5,000 rpm).**
   At least, 4,000 x g is required for proper DNA recovery.
   Insufficient centrifugal force may lead to remaining of ethanol in SV column membrane, followed by poor DNA recovery.
   If the SV column has Buffer TW associated with it after centrifugation, incubate the SV column for 10~15 min at room temperature to evaporate residual ethanol from Buffer TW. Residual ethanol can inhibit some downstream reactions, such as PCR.

10. **Place the SV column into a new 50 ml conical tube (provided). Pipet 600 μl of Buffer AE or distilled water onto a center of membrane and close the cap. Incubate for 2 min at room temperature. Centrifuge at 4,500 x g (5,000 rpm) for 5 min.**
    Lower centrifugal force will dramatically reduce the volume of eluate.
    Before this elution step, it is strongly recommended that any residual ethanol originated from Buffer TW should not remain in SV column membrane. It can be checked by smell. Residual ethanol disturbs proper DNA preparation as follows; poor DNA recovery, low purity, inhibition of subsequent reactions and etc.
    Ensure that the Buffer AE or distilled water is dispensed directly onto the center of the SV column membrane for optimal elution of DNA.
    Do not reduce the elution volume below 400 μl.
    For long-term storage, eluting in Buffer AE is recommended. But, EDTA included in the Buffer AE may inhibit subsequent enzymatic reactions, so you can avoid such latent problems by using distilled water (>pH 7.0) or Tris-Cl (>pH 8.5). When using water for elution, make sure the pH of water (at least 7.0) before elution.

11. **For higher concentrated yield, re-load the eluate from step 11 into the SV column, close the cap, incubate 2 min at room temperature, and centrifuge at 4,500 x g (5,000 rpm) for 5 min.**
    For higher total yield, add 600 μl of fresh Buffer AE or distilled water again into the SV column, close the cap, incubate 2 min at room temperature, and centrifuge at 4,500 x g (5,000 rpm) for 5 min.
    Less than 600 μl of eluate will be obtained from 600 μl of elution buffer, but this has no influence on DNA yields.

B. PROTOCOL FOR CELLS

Before experiment

- Prepare the water bath to 64°C.
- Equilibrate Buffer AE to room temperature.
- All centrifugation should be performed at room temperature.
- Buffer TB may precipitate at cold ambient temperature.
- If so, completely dissolve it in 37°C water bath.

Important Note!

All centrifugation steps MUST be carried out on a tabletop centrifuge which has an ability of 4,000 x g at least and a swing-out bucket.
DO NOT USE A FIXED-ANGLE ROTOR.

1. **Harvest cells listed below to a 15 ml conical tube by centrifugation at 2,000 x g for 15 min.**
   Cultured cells or lymphocytes : up to 5 x 10^7
   Bacterial cells : up to 2 x 10^{10}

2. **Discard the supernatant as much as possible and thoroughly resuspend cell pellet in 3 ml of Buffer TL.**
   For efficient resuspending in Buffer TL, it is helpful to loosen and resuspend the pellets by flickering or vortexing before the addition of Buffer TL.

3. **Add 200 µl of Proteinase K solution. Mix completely by vortexing or pipetting. Incubate for 20 min at 64°C.**
   Longer incubation will not affect DNA recovery.

4. **Continue with Animal Tissue Protocols from step 3 (page 35).**

C. PROTOCOL FOR MAMMALIAN WHOLE BLOOD *(PLUS! ONLY)*

* For convenient preparation from whole blood, use Exgene™ Blood SV kit.

Before experiment

- *Prepare the water bath to 64°C.*
- *Equilibrate Buffer AE to room temperature.*
- *All centrifugation should be performed at room temperature.*
- *Buffer TB may precipitate at cold ambient temperature.*
- *If so, dissolve it in 37°C water bath.*

Important Note!

All centrifugation steps MUST be carried out on a tabletop centrifuge which has an ability of 4,000 x g at least and a swing-out bucket.
DO NOT USE A FIXED-ANGLE ROTOR.

1. **Transfer 5 ml of whole blood to a new 50 ml conical tube.**
   Before transfer of blood, it is recommended that gently rocking the tube of blood until throughly mixed.

2. **Add 15 ml of Buffer RL to the tube containing the blood sample. Invert the tube 5-6 times to mix. Incubate the mixture for 10 min at room temperature.**
   Invert 2-3 times during the incubation. The lysate should become clear (translucent). If the lysate is turbid (opaque) not clear, it may be frozen or mis-stored sample, and you should read the annotation of step 3.
   For simple and convenient preparation from blood samples, we recommend Exgene™ Blood SV.

3. **Centrifuge for 15 min at 2,000 x g and carefully remove the supernatant as much as possible without disturbing the visible white (or pink) pellet. Resuspend the pellet by vortexing or flickering.**
   A little of residual liquid will remain. Steps 2-3 are critical steps for DNA recovery yields, so you have to check the translucent lysate and the white (or pink) pellet before processing next steps.
   If blood sample has been frozen or mis-stored, resuspend the pellet and repeat step 2-3 with the sample until the lysate becomes translucent.

4. **Add 3 ml of Buffer TL to the tube containing the resuspended cells. Pipet the solution 5-6 times to mix well.**
   The lysate may be viscous.

5. **Add 150 µl of Proteinase K solution. Mix thoroughly by vortexing. Incubate for 20 min at 64˚C.**

6. **Continue with Animal Tissue Protocols from step 3 (page 35).**

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low or no recovery	**Too much starting material**	Too much starting material brings about inefficient lysis and/or SV column clogging, followed by poor DNA yields. Reduce the amount of starting material.
	Starting material is too old or mis-stored	Best results will be obtained with fresh sample. DNA yield is dependent on the type, size, age and storage of starting material. Lower yields will be obtained from material that has been inappropriately stored. For example, blood samples that have been stored at 4°C for more than 5 days may give reduced yields.
	Low cells in the sample	Low cell-density of starting sample leads to poor result. Increase the sample amount and load the SV column several times. Reduce the elution volume to 50 μl. If possible, harvest fresh sample and repeat the DNA purification with a new sample.
	Cell pellet was not resuspended thoroughly in step 2 of cultured cell protocol or step 3 of Blood protocol	For proper cell lysis with Buffer TL, it is essential to resuspend thoroughly the cell pellet. Inefficient cell lysis leads to many problems including poor DNA yields.
	Improper centrifuge (Midi/MAXI)	Swing-bucket rotor must be used instead of fixed angle rotor.
	G-force in the protocol was not reach to 4,000 x g (Midi/MAXI)	For proper DNA purification, centrifugal g-force in washing step must reach 4,000 x g at least (page 11).

Facts	Possible Causes	Suggestions
Low or no recovery	**Insufficient lysis**	Incomplete lysis is due to too much starting material, imperfect mixing with Buffer TL, insufficient time to lyse completely or poor disruption of sample. In next purification, check it carefully.
	Weaken activity of Proteinase K caused by mis-storage or out-of-date	Proteinase K must be stored under 4°C for maintenance of proper activity. Lysis can not be done properly with degenerated Proteinase K. Replace with new one.
	Improper eluent	As user's need elution buffer other than Buffer AE can be used. However, the condition of optimal elution should be low salt concentration with alkaline pH (7<pH<9). When water or other buffer was used as eluent, ensure that condition.
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 TL, insufficient time to lyse completely, or poor disruption of sample. Check these out in next preparations.
Low concentration of DNA in eluate	**Low cells in starting material or small starting material used**	Increase the amount of starting material with additional volume of buffer. Otherwise reduce the elution volume to the minimum and/or do re-elution with eluate.
Column clogging	**Insufficient lysis**	Insufficient lysis may lead to column clogging. In next preparations, mix the sample with each buffer completely, reduce the starting materials, extend the incubation time at 56°C or increase the amount of Proteinase K to double.

Facts	Possible Causes	Suggestions
White precipitate in Buffer TB	**Buffers were stored in cool ambient condition**	Storage at low temperature may cause precipitation in Buffer TB. For proper DNA purification, any precipitate in the buffer should be dissolved completely by incubating the buffer at 37°C (or above) until it disappears.
Enzymatic reaction is not performed well with purified DNA	**High salt concentration in eluate**	Ensure that all washing steps were carried out just in accordance with the protocols. Additional washing with Buffer TW helps usually remove high salt in eluate. Refer the annotation of elution step.
	Low purity of DNA	Check "Low A_{260}/A_{280} ratio"
Degraded DNA	**Starting material is too old or mis-stored**	Too old or mis-stored sample often yield degraded DNAs. Use fresh sample.
	Residual ethanol from Buffer TW remains in eluate	Ensure that wash steps in protocol is performed properly. SV column membrane should be completely dried via additional centrifugation or air-drying.

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*

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
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	

Products	Scale	Size	Cat. No.	Type

GeneAll® Protein series

ProtinEx™ Animal cell / tissue	100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001	solution

GeneAll® STEADi™ *for automatic nucleic acid purification*

12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell / Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Total RNA	96	404-304	kit
Viral DNA / RNA	96	405-322	kit
Genomic DNA Plant	96	407-117	kit
Soil DNA	96	408-114	kit

GeneAll® GENTi™ 32 *Ultimately flexible automatic extraction system*

Automatic extraction equipment		GTI032	system
Genomic DNA	48	901-048	tube
	96	901-096	plate
Viral DNA / RNA	48	902-048	tube
	96	902-096	plate
Whole Blood Genomic DNA	48	903-048	tube
	96	903-096	plate

Products	Scale	Size	Cat. No.	Type

GeneAll® GENTi™ 32 ADVANCED *Ultimately flexible automatic 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
Plant DNA / RNA	48	904-048A	tube
	96	904-096A	plate

memo

memo

memo

GeneAll® Exgene™ Tissue

Brief mini protocol for an animal tissue

** Before use this protocol, we strongly recommend you first read carefully the detailed protocol at page 13*

Disruption and lysis

1. Disrupt 20 mg of tissue sample in 200 μl of Buffer TL by homogenization, grinding or mincing.
2. Add 20 μl of Proteinase K and mix well.
3. Incubate at 56°C for complete lysis.
4. *(Optional)* For RNA-free DNA, treat 4 μl of RNase A (100 mg/ml) and incubate for 2 min at RT.

Binding

5. Add 400 μl of Buffer TB and mix well.
6. Apply the mixture into SV column.
7. Centrifuge for a min and replace the collection tube with new one.

Washing

8. Apply 600 μl of Buffer BW into SV column.
9. Centrifuge for 30 sec and replace the collection tube with new one.
10. Apply 700 μl of Buffer TW into SV column.
11. Centrifuge for 30 sec, discard the filtrate and re-insert the collection tube back.
12. Centrifuge for 1 min at full speed.

Elution

13. Place the SV column into a fresh 1.5 ml microcentrifuge tube.
14. Add 200 μl of Buffer AE, incubate for 2 min at RT and centrifuge for 1 min at full speed.

GeneAll® Exgene™ Tissue

Brief Midi (MAXI) protocol for an animal tissue

** Before use this protocol, we strongly recommend you first read carefully the detailed protocol at page 26 or 34*

** The number in the parenthesis denote the amounts for MAXI kit*

Disruption and lysis

1. Disrupt up to 100 (250) mg of tissue sample in 1 (3) ml of Buffer TL by homogenization, grinding or mincing.

2. Add 100 (250) μl of Proteinase K and mix well.

3. Incubate at 64°C for complete lysis.

4. *(Optional)* For RNA-free DNA, treat 15 (40) μl of RNase A (100 mg/ml) and incubate for 3 min at RT.

Binding

5. Add 2 (7) ml of Buffer TB and mix well.

6. Apply the mixture into SV column.

7. Centrifuge for 2 min and discard the filtrate.

Washing

8. Apply 3 (7) ml of Buffer BW into SV column.

9. Centrifuge for 2 min and discard the filtrate.

10. Apply 4 (10) ml of Buffer TW into SV column.

11. Centrifuge for 15 min at full speed.

Elution

12. Place the SV column into a fresh 15 (50) ml conical tube.

13. Add 300 (600) μl of Buffer AE, incubate for 2 min at RT and centrifuge for 5 min at full speed.

GeneAll

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