Ver 3.2

Handbook for

CELL SV MINI
CLINIC SV MINI
BLOOD SV MINI

exgene™

DNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

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This protocol handbook is included in :

GeneAll® Exgene™ Blood SV mini (105-101, 105-152)
GeneAll® Exgene™ Clinic SV mini (108-101, 108-152)
GeneAll® Exgene™ Cell SV mini (106-101, 106-152)

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

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KIT CONTENTS

	Blood SV mini		Clinic SV mini	
Cat. No.	**105-101**	**105-152**	**108-101**	**108-152**
Size	**mini**	**mini**	**mini**	**mini**
No. of preparation	100	250	100	250
Mini column type G	100	250	100	250
Collection tube	300	750	300	750
Buffer CL	-	-	25 ml	60 ml
Buffer BL	25 ml	60 ml	25 ml	60 ml
Buffer BW	80 ml	200 ml	80 ml	200 ml
Buffer TW	100 ml	250 ml	100 ml	250 ml
Buffer AE*	30 ml	60 ml	30 ml	60 ml
Proteinase K (mg)**	48 mg	120 mg	48 mg	120 mg
PK Storage bfr. (ml)	4 ml	7 ml	4 ml	7 ml
Protocol Handbook	1	1	1	1

	Cell SV mini	
Cat. No.	**106-101**	**106-152**
Size	**mini**	**mini**
No. of preparation	100	250
Mini column type G	100	250
Collection tube	300	750
Buffer GP	25 ml	60 ml
Buffer YL	60 ml	125 ml
Buffer CL	25 ml	60 ml
Buffer BL	25 ml	60 ml
Buffer BW	80 ml	200 ml
Buffer TW	100 ml	250 ml
Buffer AE*	30 ml	60 ml
Proteinase K (mg)**	48 mg	120 mg
PK Storage bfr. (ml)	4 ml	7 ml
Protocol Handbook	1	1

** 10mM Tris-HCl, pH 9.0, 0.5mM EDTA*

*** After reconstitution of proteinase K, store it 4˚C or -20˚C*

STORAGE CONDITION

All components of GeneAll® Exgene™ series should be stored at room temperature (15 ~ 25°C). 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 can be formed in buffer BL or CL. Heat the bottle at 56°C to dissolve completely in such a case. Using precipitated buffers will lead to poor DNA recovery.

GeneAll® Exgene™ series are guaranteed until the expiration date printed on the product box.

QUALITY CONTROL

All components in GeneAll® Exgene™ series 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™ series 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 BL 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.

INTRODUCTION

GeneAll® Exgene™ series including Blood, Clinic, and Cell SV kit provide fast and easy methods for the small scale purification of total DNA from various sample species, such as blood, tissues, bacterial or cultured cells, and forensic specimens. Purified DNA can be used directly for PCR, Southern blotting, and other downstream applications.

GeneAll® Exgene™ Series utilize the advanced silica-binding technology to purify total DNA sufficiently pure for many applications. Various samples are lysed in optimized buffer containing detergents and lytic enzyme. Under high salt condition, DNA in the lysate bind to silica membrane and impurities pass through membrane into a collection tube. The membranes are washed with a series of alcohol-containing buffer to remove any traces of proteins, cellular debris and salts. Finally pure DNA is released into a clean collection tube with deionized water or low ionic strength buffer.

This protocol can be used with:

- GeneAll® Exgene™ Blood SV mini (105-101/105-152)
  Fresh or frozen blood, body fluid, nucleated blood, lymphocyte, cultured cells, buccal swab, saliva, hair, sperm and etc.

- GeneAll® Exgene™ Clinic SV mini (108-101/108-152)
  (In addition to Blood SV's) Fresh, frozen or fixed animal tissue, dried blood spot, gram-negative bacteria and etc.

- GeneAll® Exgene™ Cell SV mini (107-101/107-152)
  (In addition to Clinic SV's) Gram positive bacteria, yeast and etc.

GENERAL CONSIDERATION

Sample amount and expected yield

GeneAll® Exgene™ series is designed for preparation from small amount of starting sample. Starting sample amount should not exceed the recommended maximum limit, otherwise DNA recover will be significantly lowered. (Fig.1) Recommended amount of starting sample and the yield is listed on next page. For samples with very high DNA contents (e.g., buffy coat, spleen, which has a high cell density, and cell lines with a high degree of ploidy), less than the recommended should be used.

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.

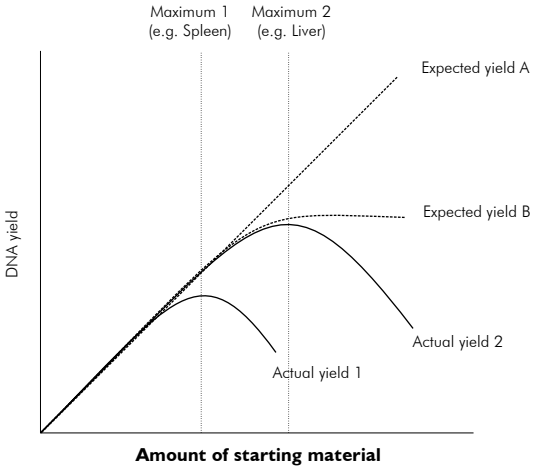


Fig. 1 The amount of starting sample should not be exceed the recommended maximum limit, otherwise DNA recover will be significantly low. If the cell mass of starting material is high (e.g., spleen, actual yield 1), maximum capacity will be lowered. (Maximum 1)

The DNA yield from whole blood will depend on the number of white blood cells (WBCs, leukocytes) contained in the sample. The number of WBCs varies from sample to sample, and can be determined using hematocytometer or other cell counter before experiment. This kit can be used to extract total DNA from blood containing as little as 2.5×10^5 leukocytes per milliliter and up to 1×10^7 cells per milliliter.

Generally, the density of bacterial cells can not be easily determined, because its optical density is influenced by various factors, such as species, media and measuring devices. Rough guide may be helpful with the bacterial cells. $A_{600}=1$ corresponds to 1×10^9 cells per milliliter with *E.coli*. For yeast, $A_{600}=1$ is obtained with a cell density of $1 \sim 2 \times 10^7$ cells per milliliter.

Sample	Starting amount (max. capacity)	Yield (ug)
Whole blood	200 ul	3 ~ 12
Buffy coat	200 ul	20 ~ 40
Nucleated blood	10 ul	5 ~ 16
Cultured cells or lymphocytes	5×10^6	14 ~ 25
Brain	20 mg	5 ~ 18
Heart	20 mg	4 ~ 10
Kidney	20 mg	15 ~ 35
Liver	20 mg	15 ~ 35
Lung	20 mg	4 ~ 10
Pancreas	20 mg	8 ~ 25
Spleen	10 mg	10 ~ 35
Bacteria	2×10^9	5 ~ 25
Yeast	5×10^7	10 ~ 25

Table 1 The yield on 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 this data.

For preparation of DNA from larger size of starting materials than the recommended above, we recommend GeneAll® Exgene™ Midi or MAXI series which is capable of processing the larger samples; On average, 4 times (Midi) and 10 times (MAXI) to mini series. (See ordering information at page 42)

Sample preparation

The yield and purity of DNA can be varied depending on the methods for harvesting and/or storing the starting sample materials. Freshly harvested sample should be used or stored immediately for best result. Note that the sample should be handled on ice as quickly as possible and repeated freezing and thawing of frozen sample should be avoided.

Blood and its derivatives

Blood sample should be used or stored immediately after collected to the tubes containing the anticoagulants and the preservatives for whole blood. Whole blood collected in anticoagulants, such as EDTA or citrates (CPDs and ACDs), can be stored for several days at 4°C and at least for 2 years at -80°C without significant change in its properties. EDTA, a metal chelator, is an inhibitor against metal-dependent nuclease and is most preferable anticoagulant for DNA preparation. Heparin can also be used as anticoagulant but is not usually used because it acts as an inhibitor in PCR reaction. Frozen blood should be thawed quickly in 37°C water bath and kept on ice before use.

The derivatives, such as plasma, serum or buffy coat, can also be used for specific application. Buffy coat can be used for higher yield of DNA and is prepared by collecting the intermediate phase after the centrifugation of whole blood.

150 ~ 250 ul of buffy coat can be collected from 3 ml of whole blood, but the concentration of leukocytes should be determined because overload of leukocyte will lead to poor result. If the number of leukocytes exceeds 5×10^6, DNA recovery will be significantly decreased.

Cultured cells

Cultured cells growing in suspension can be easily harvested by centrifugation. However attached cells should be treated with trypsin-EDTA for detaching the cells before harvesting. The number of cells should be determined using a hematocytometer or other cell counter, and should not be over 5×10^6 per prep. Harvested cells washed with phosphate buffered saline (PBS) can be used directly in DNA preparation or stored at -20°C or -80°C in pellet. It is not recommended washing the fixed cells with PBS, because it can cause cell lysis and significant reduce in DNA yield. Sample should always be kept on ice before use.

Tissue

Harvested tissues should be used freshly or stored at very low temperature as quickly as possible. Generally, to make the sample finer will lead to not only better result, but also economy of experiment-time. Grinding in mortal and pestle under liquid nitrogen is a good method for disrupting the sample, but alternative methods, such as a homogenizer or a bead-beater, can be employed in case by case for efficient disruption. Shaking or vortexing during incubation for lysis may greatly accelerate the efficiency of lysis, resulting in reduced time for complete lysis. Note that the freshness and the particle size of disrupted sample is the key for good result and that the frozen sample should be kept on ice until use.

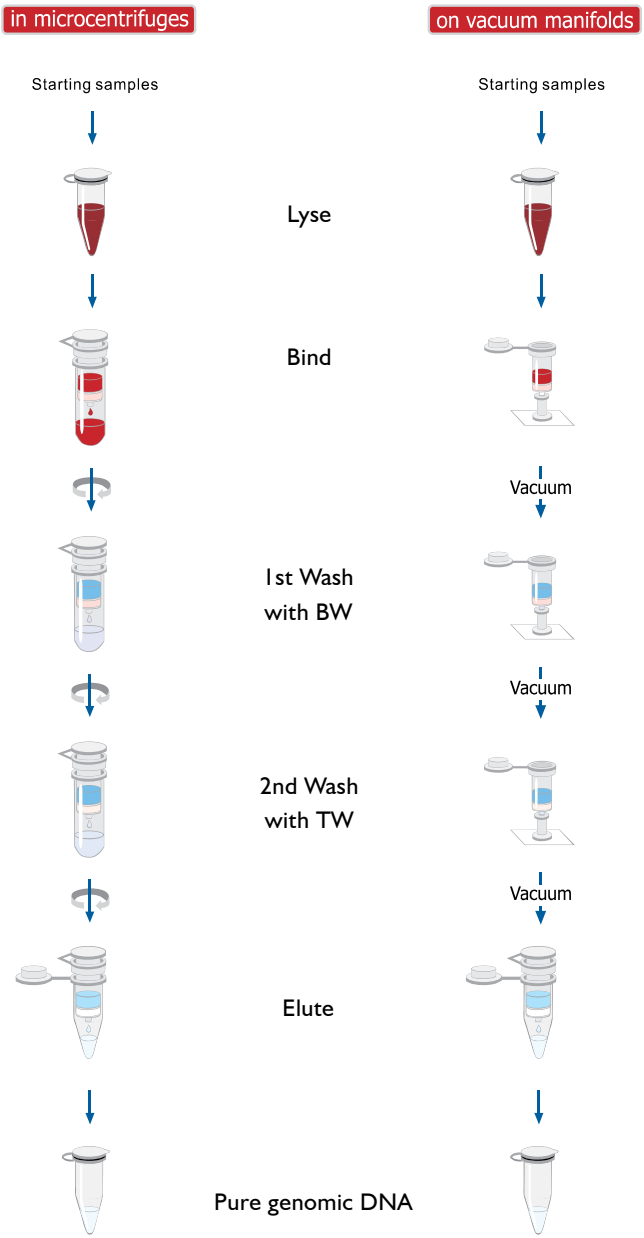
Bacterial cells

Bacterial cells can be prepared by incubating the culture for 12 ~ 24 hours at 37°C with vigorous shaking until the cell reach the log phase. Harvested bacterial cells can be used directly or stored at -20°C or -80°C for future use. Gram positive bacteria should be treated with lysozyme or lysostaphin to weaken the rigid and multilayered cell wall, while gram negative does not need to. Extreme care should be taken for pathogenic bacteria.

Yeast cells

Yeast cells are troublesome for purification of DNA, because its rigid cell wall does not lysed well in usual lytic condition. The cell wall of yeast should be loosened by enzymatic lysis with an lytic enzyme such as lyticase or zymolase, and spheroplasts are then collected by centrifugation. This harvested spheroplasts can be used directly for DNA preparations or stored at -20°C or -80°C for later use. When the value of A_{600} is 1, the cell density of yeast culture may be 1 ~ 2 x 10^7 cells per milliliter.

KIT PROCEDURES



Elution

Purified DNA is eluted from SV column membrane in either deionized water or buffer AE which contains 0.5 mM EDTA and 10 mM Tris-HCl, pH9.0. Elution buffer should be equilibrated to room temperature before applying to SV column.

Typically, elution is carried out in two successive steps using 200 ul 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 ul per column) and should not be over 300 ul. Basically, it is recommended for the 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. Yield may be slightly increased if the SV column is incubated with the elution buffer at room temperature for 5 min before centrifugation.

Generally, DNA bound to the SV column will not be eluted completely with a single elution step. Approximately 60 ~ 85 % of the DNA will elute in the first 200 ul, and the rest of bound DNA in next 200 ul. (Fig.2) However, a single elution with 200 ul of elution buffer will be sufficient to recover the amount of DNA required for multiple PCR reactions. For very small samples (containing less than 1ug of DNA), only a single elution in 50 ul of buffer AE or deionized water is recommended.

The SV column for GeneAll® Exgene™ series co-purifies DNA and RNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, although it does not inhibit PCR itself. If RNA-free DNA is required, RNase A should be treated at the optional step included in each protocol. A treatment of RNase A will decrease the overall yield measured by spectrophotometer, but the virtual recovery of DNA will be slightly increased. RNase A can be purchased at GeneAll Biotechnology (www.geneall.com), but any equivalent can be used at the concentration of 20 mg/ml.

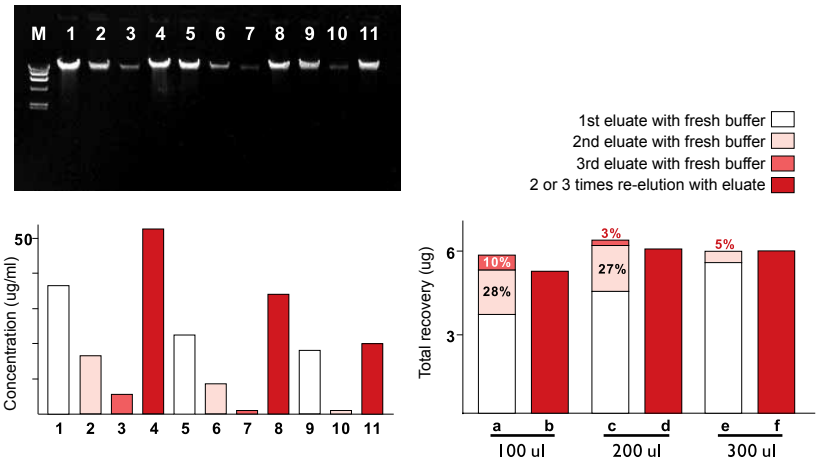
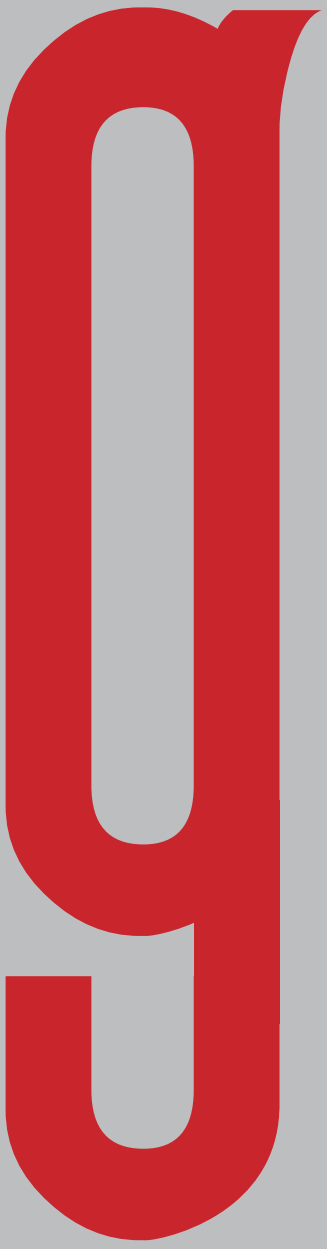


Fig. 2 The yield and concentration of purified DNA depending on the elution method.
DNA was prepared from 200 ul of bovine whole blood. Each preparation was exactly identical except the elution method; Elution was performed 3 times per column with 100 ul (lane 1 ~ 3) and 200 ul (lane 5 ~ 7), and 2 times per column with 300 ul (lane 9 ~ 10) of fresh buffer AE. At the same time, another elution was carried out 3 times (100 ul and 200 ul) and 2 times (300 ul) by recursive use of the eluate instead of fresh buffer AE. (lane 4, 8, 11) Total 11 eluates purified from 6 samples were resolved on 0.8 % agarose gel to visualize (upper left) and its concentration (lower left) and total yield (lower right) was measured by spectrophotometric analysis.

GeneAll® Exgene™ PROTOCOLS

Read the protocol carefully before experiment.



A. PROTOCOL FOR BLOOD AND BODY FLUID USING MICROCENTRIFUGE

Before experiment

- *Prepare the water bath to 56°C*
- *Prepare absolute ethanol*
- *Prepare 1.5 ml microcentrifuge tube*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *If a precipitate has formed in buffer BL, heat to dissolve at 56°C before use*

This protocol can be used with Blood/Clinic/Cell SV mini

1. Pipet 20 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml tube.

If the sample volume is larger than 200 ul, increase the amount of Proteinase K proportionally.

When the cell mass is low, up to 400 ul of starting sample can be used. For 400 ul of sample volume, 40 ul of Proteinase K solution is needed.

2. Transfer 200 ul of sample to the tube. Use the starting sample listed below.

If the sample volume is less than 200 ul, adjust the volume to 200 ul with 1x PBS.

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

3. ***(Optional :)*** **If RNA-free DNA is required, add 20 ul of RNase solution (20 mg/ml, Cat. No. 391-001) to the sample, pipet 2 ~ 3 times to mix and incubate for 2 min at room temperature.**

Unless RNase A is treated, RNA will be copurified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 ul of Buffer BL 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 ul, increase the volume of buffer BL in proportion. Ratio of buffer BL to the starting sample volume is 1:1.
It is essential to mix the sample and buffer BL thoroughly for good result.
Longer incubation will not affect DNA recovery.

5. **Add 200 ul of absolute ethanol (not provided) to the sample, Pulse-vortex 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 ul, increase the ethanol volume proportionally.

6. **Transfer the mixture to the SV column carefully, centrifuge for 1 min at 6,000 xg above (>8,000 rpm), and replace the collection tube with new one (provided).**

If starting sample volume is larger than 200 ul, apply the mixture twice; apply 700 ul 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 SV column.

If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13,000 xg) until all of the solution has passed through. Centrifugation at full speed is recommended to avoid clogging especially when applying the sample with high-cell density, such as buffy coat, lymphocyte or cultured cells. Centrifugation at full speed will not affect DNA recovery.

7. **Add 600 ul of Buffer BW, centrifuge for 1 min at 6,000 xg above (>8,000 rpm) and replace the collection tube with new one (provided).**
If the SV column has colored residue after centrifuge, repeat this step until no colored residue remain. See Trouble shooting guide for detail.
Centrifugation at full speed will not affect DNA recovery.

8. **Apply 700 ul of Buffer TW. Centrifuge for 1 min at 6,000 xg above (>8,000 rpm). Discard the pass-through and reinsert the SV column back into the collection tube.**
Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed 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 a carryover of buffer TW still occurs, centrifuge again for 1 min at full speed with the collection tube before transferring to a new 1.5 ml tube.
Centrifugation must be performed at full speed (13,000 xg ~ 20,000 xg).

10. Add 200 ul of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.

** For low cell-density sample, such as body fluids or virus, use 50 ~ 150 ul elution buffer as based on the species and conditions of starting sample or the downstream applications.*

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

Repeat of elution step with fresh 200 ul elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 ul 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 ul 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 first eluate instead of fresh elution buffer. Alternatively for higher concentration, elution volume can be decreased to 50 ul. 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 can inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled deionized water (>pH7.0) or Tris-HCl (>pH8.5). *When using water for elution, check the pH of water before elution.*

B. PROTOCOL FOR BUCCAL SWAB

Before experiment

- *Prepare the water bath to 56°C*
- *Prepare sterile sharp blade (or wire cutter) and tweezer*
- *Prepare 1x PBS and absolute ethanol*
- *Prepare 1.5 ml microcentrifuge tube*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in buffer BL, heat to dissolve at 56°C*

* Due to the need of additional buffer BL for buccal swab, fewer preparations can be performed. Buffer BL can be purchased separately as accessory. (105-901)

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 who provides the sample has not taken any food or drink in 30 min prior to sample collection.

2. **Place the swab in 2 ml sterilized microcentrifuge tube. Clip off handle of brush with sterile sharp blade or wire cutter. Add 400 ul 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 ul of RNase A (20 mg/ml), vortex to mix, and incubate 2 min at room temperature.**
   Unless RNase A is treated, RNA will be co-purified with DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Apply 20 ul of Proteinase K (20 mg/ml) and 400 ul of Buffer BL to the sample. Vortex vigorously to mix immediately.**
   For efficient lysis, mix the sample completely.

5. **Incubate at 56˚C for 10 min. Briefly centrifuge to remove any drops from inside the lid.**

6. **Add 400 ul of absolute ethanol to the lysate, and mix well by vortexing. Briefly centrifuge to remove any drops from inside the lid.**

7. **Transfer carefully up to 700 ul of the mixture to the SV column. Close the cap. Centrifuge for 1 min at 6,000 xg above (>8,000 rpm). Discard the pass-through and reinsert the SV column back into the collection tube.**
   Be careful not to wet the rim of the SV column.

8. **Repeat step 7 until all the remaining mixture has been applied to the SV column. Replace the collection tube with new one (provided).**

9. **Continue with step 7 in Blood and body fluid protocol (page 17).**

C.

PROTOCOL FOR
SALIVA AND MOUTHWASH

Before experiment

- *Prepare the water bath to 56°C*
- *Prepare 1.5 ml microcentrifuge tube and 50 ml conical tube*
- *Prepare 1x PBS (Phosphate buffered saline) and absolute ethanol*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in buffer BL, heat to dissolve at 56°C*

1. **Collect 10 ml of mouthwash in a 50 ml conical tube, or collect 1 ml of saliva by spitting in a 50 ml conical tube. If saliva is used, add 5 ml of 1x PBS to the sample and vortex to mix.**
   To avoid contamination from other materials, ensure that the person who provides the sample has not taken any food or drink in the 30 min prior to sample collection.

2. **Centrifuge at 2,000 xg (3,000 rpm) for 5 min to pellet cells. Immediately and carefully decant the supernatant to prevent loose cell pellets. Resuspend completely the pellets in 200 ul of 1x PBS.**
   If the pellets are loose, repeat centrifugation.

3. ***(Optional :)*** **If RNA-free DNA is required, add 20 ul of RNase A (20 mg/ml), vortex to mix, and incubate 2 min at room temperature.**
   Unless RNase A is treated, RNA will be copurified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Apply 20 ul of Proteinase K (20 mg/ml) and 200 ul of Buffer BL to the sample. Vortex vigorously to mix completely.**
   For efficient lysis, mix the sample completely.

5. **Continue with step 4 in Blood and body fluid protocol (page 16).**

This protocol can be used with Blood/Clinic/Cell SV mini

D.

PROTOCOL FOR
HAIR

Before experiment

- *Prepare the water bath to 56 °C*
- *Prepare 1.5 ml microcentrifuge tube*
- *Prepare absolute ethanol*
- *Prepare **buffer H** as follow;*
  10 mM Tris-HCl, pH8.0, 10 mM EDTA, 100 mM NaCl, 2 % SDS, 40 mM DTT (Add DTT immediately before use, because it oxidizes quickly in aqueous solutions.)
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in buffer BL, heat to dissolve at 56 °C*

1. **Collect hair sample in a 1.5 ml microcentrifuge tube.**
   The amount of starting sample should not exceed 30 mg. It is recommended to use 0.5 ~ 1 cm from the root ends of plucked hair samples.

2. **Add 180 ul of prepared Buffer H and 20 ul of Proteinase K to the tube, and vortex to mix thoroughly.**

3. **Incubate at 56°C for at least 1 hour until the sample is dissolved. Spin down briefly to remove any drops from inside of the lid.**
   Invert the tube occasionally to disperse the sample, or place on a rocking platform. Hair follicles should be completely dissolved, however hair shaft may be not dissolved completely and this residual solid materials will not affect DNA recovery.

4. **Continue with step 3 in Blood and body fluid protocol (page 16).**

E. PROTOCOL FOR SPERM

Before experiment

- *Prepare the water bath to 56 °C*
- *Prepare 1.5 ml microcentrifuge tube*
- *Prepare absolute ethanol*
- *Prepare **buffer H2** as follow;*
  20 mM Tris-HCl, pH8.0, 20 mM EDTA, 200 mM NaCl, 4 % SDS, 80 mM DTT (Add DTT immediately before use, because it oxidizes quickly in aqueous solutions.)
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature.*
- *If precipitate has formed in buffer BL, heat to dissolve at 56 °C*

This protocol can be used with Blood/Clinic/Cell SV mini

1. **Place 100 ul sperm in a 1.5 ml microcentrifuge tube. Add 100 ul of Buffer H2 and 20 ul of Proteinase K to the tube. Mix thoroughly by vortexing.**

2. **Incubate at 56 °C until the sample is dissolved completely. Spin down briefly to remove any drops from inside of the lid.**
   It may need at least 1 hour for complete lysis.
   Invert the tube occasionally to disperse the sample, or place on a rocking platform.

3. **Continue with step 3 in Blood and body fluid protocol (page 16).**

F.

PROTOCOL FOR
BLOOD AND BODY FLUID USING VACUUM

Before experiment

- *Prepare the water bath to 56 °C*
- *Prepare absolute ethanol*
- *Prepare 1.5 ml microcentrifuge tube*
- *Prepare vacuum system ; manifold, trap, tubing and pump capable of 15 ~ 20 inchHg*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *If a precipitate has formed in buffer BL, heat to dissolve at 56 °C before use*

Blood/Clinic/Cell SV mini

This protocol can be used with

1. Pipet 20 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml tube.

If the sample volume is larger than 200 ul, increase the amount of Proteinase K proportionally.

When the concentration of cells is low, up to 400 ul of starting sample can be used. For 400 ul of sample volume, 40 ul of Proteinase K solution is needed.

2. Transfer 200 ul of sample to the tube. Use the starting sample listed below.

If the sample volume is less than 200 ul, adjust the volume to 200 ul with 1x PBS.

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

3. ***(Optional :)*** **If RNA-free DNA is required, add 20 ul of RNase solution (20 mg/ml, Cat. No. 391-001) to the sample, pipet 2 ~ 3 times to mix and incubate for 2 min at room temperature.**

   Unless RNase A is treated, RNA will be copurified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 ul of Buffer BL 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 ul, increase the volume of buffer BL in proportion. Ratio of buffer BL to the starting sample volume is 1:1.

   It is essential to mix the sample and buffer BL thoroughly for good result.

   Longer incubation will not affect DNA recovery.

5. **Add 200 ul of absolute ethanol (not provided) to the sample, Pulse-vortex 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 ul, increase the ethanol volume proportionally.

6. **Attach the SV column to a port of the vacuum manifold tightly. If available, use vacuum adaptors to avoid cross-contamination between the samples.**

   Most commercial vacuum manifold with luer connectors can be adopted to this protocol.

   If the SV column becomes clogged during this procedure, it is possible to switch to the procedure for purification by centrifugation (page 16).

7. **Transfer the mixture to the SV column by pipetting. Switch on vacuum source to draw the solution through the SV column. When all liquid has been pulled through the SV column, release the vacuum.**

   If starting sample volume is larger than 200 ul, repeat this step until all of mixture have applied to the SV column.

   If the mixture has not passed completely through the membrane, you can switch to centrifugation protocol by step 6 at page 16.

8. **Apply 600 ul of Buffer BW and switch on vacuum source. When all liquid has been pulled through the SV column, release the vacuum.**
If the SV column has colored residue after this step, repeat this step until no colored residue remain. See Trouble shooting guide for detail.

9. **Apply 700 ul of Buffer TW and switch on vacuum source. When all liquid has been pulled through the SV column, release the vacuum. Transfer the SV column into a empty collection tube (Provided).**

10. **Continue with step 9 in Blood and body fluid centrifugation protocol (page 17).**

G.

PROTOCOL FOR
ANIMAL TISSUE

Before experiment

- *Prepare the water baths or incubators to 56°C and 70°C*
- *Prepare absolute ethanol*
- *Prepare 1.5 ml microcentrifuge tube*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *Buffer BL and CL may precipitate at cool ambient temperature If so, dissolve it in 56°C water bath*

1. Homogenize up to 20 mg of tissue as described in step 1a, 1b or 1c, depending on the sample type.

To disrupt the sample finer will accelerate lysis and decrease the lysis time. For spleen tissue, up to 10 mg can be processed.

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

Carefully homogenize for minimization of foaming.

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 20 mg of the powdered tissue into 1.5 ml tube. Add 200 ul of Buffer CL and pulse-vortex for 15 sec.

1c. If neither 1a nor 1b is available, mince the tissue with sharp blade or scalpel as small as possible. Put the tissue into a 1.5 ml tube. Add 200 ul of Buffer CL 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.***

This protocol can be used with Clinic/Cell SV mini

2. **Add 20 ul of Proteinase K solution. Mix completely by vortexing or pipetting. Incubate at 56˚C until the sample is completely lysed. Spin down the tube briefly to remove any drops from inside of the lid.**
   It is essential to mix the components completely for proper lysis.
   Lysis time varies from 10 min to 3 hrs usually depending on the type of tissue and the homogenization method (step1). The lysate should become translucent without any particles after complete lysis. Overnight lysis does not influence the preparation.
   If the sample is lysed 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. ***(Optional:)* If RNA-free DNA is required, add 20 ul of RNase solution (20 mg/ml, Cat.No.391-001), vortex to mix thoroughly, and incubate for 2 min at room temperature.**
   Unless RNase A is treated, RNA will be copurified with DNA, especially when using transcriptionally active tissues, such as liver and kidney. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 ul of Buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 70˚C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   Cool down to room temperature before proceeding.
   It is important to mix the sample and buffer BL thoroughly for good result.

5. **Add 200 ul of absolute ethanol (not provided) to the sample, pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
   It is important to mix the sample and ethanol completely for good result.
   After addition of ethanol, a white precipitate may be formed. It is essential to apply all of the mixture including the precipitate to the SV column on next step.

6. **Transfer all of the mixture to the SV column carefully, centrifuge for 1 min at 6,000 xg above (>8,000 rpm), and replace the collection tube with new one (provided).**
   If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13,000 xg) until all of the solution has passed through. Centrifugation at full speed will not affect DNA recovery.

7. **Add 600 ul of Buffer BW, centrifuge for 1 min at 6,000 xg above (>8,000 rpm) and replace the collection tube with new one (provided).**
   If the SV column has colored residue after centrifuge, repeat this step until no colored residue remain. See Trouble shooting guide for detail.
   Centrifugation at full speed (>13,000 xg) will not affect DNA recovery.

8. **Apply 700 ul of Buffer TW. Centrifuge for 1 min at 6,000 xg above (>8,000 rpm). Discard the pass-through and reinsert the SV column back into the collection tube.**
   Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed (>13,000 xg) for 1 min to remove residual wash buffer. Place the SV column into a fresh 1.5 ml tube (not provided).**
   Care must be taken at this step for eliminating the carryover of buffer TW.
   If a carryover of buffer TW still occurs, centrifuge again for 1 min at full speed with the collection tube before transferring to the new 1.5 ml tube.
   Centrifugation must be performed at full speed (13,000 xg ~ 20,000 xg).

10. Add 200 ul of Buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed (>13,000 xg) for 1 min.

** For the sample expected to yield a little DNA, such as paraffin-embeded, formalin-fixed tissue, or dried blood spot or sperm, it is recommended to use 50 ~ 150 ul elution buffer as based on the species and conditions of starting sample or the downstream applications.*

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

Repeat of elution step with fresh 200 ul elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 ul 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 ul 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 the starting sample amount is very small, the second elution can be carried out with the first eluate instead of fresh elution buffer. Alternatively for higher concentration, the elution volume can be decreased to 50 ul. However the small volume of elution buffer will reduce 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 (>pH7.0) or Tris-HCl (>pH8.5). *When using water for elution, check the pH of water before elution.*

H.

PROTOCOL FOR
PARAFFIN-FIXED TISSUE

Before experiment

- *Prepare xylene and absolute ethanol*
  Xylene is an irritant and appropriate precautions should be taken in handling. For example, wear gloves, safety goggles, and a laboratory coat, avoid contact with skin, eyes and clothing and work in a fume hood.
- *Prepare the water bath to 56 °C*
- *Prepare 1.5 ml microcentrifuge tube*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath*

1. **Place a small section of paraffin-fixed tissue (up to 25 mg) in a 2 mL microcentrifuge tube.**
   Minced tissue may be de-paraffinized more efficiently.

2. **Add 1200 ul xylene. Vortex vigorously until the paraffin has been completely melted. Centrifuge at full speed (>13,000 xg) for 5 min. Carefully remove supernatant by pipetting.**
   Be careful not to lose any of the pellet.

3. **Add 1200 ul of absolute ethanol to the pellet to remove residual xylene and mix by vortexing.**

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

5. **Repeat the steps 3 ~ 4 once or twice.**

6. **Evaporate the residual ethanol by incubating the microcentrifuge tube at room temperature for 10 ~ 15 min with opened cap.**

7. **Apply 180 ul of Buffer CL and mix completely by vigorous vortexing. Continue with step 2 of Tissue protocol on page 28.**

This protocol can be used with Clinic/Cell SV mini

I.

PROTOCOL FOR
ALCOHOL- OR FORMALIN-FIXED TISSUE

Before experiment

- Prepare absolute ethanol
- Prepare the water bath to 56 °C
- Prepare 1.5 ml microcentrifuge tube
- Equilibrate buffer AE to room temperature
- All centrifugation should be performed at room temperature
- Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 56 °C water bath

1. **Briefly blot excess fixative from tissue on clean absorbent paper. Place a small section of fixed tissue (up to 20 mg) in a 1.5 ml tube.**
   Minced tissue may be lysed more efficiently.

2. **Apply 400 ul of 1x PBS to the tube. Vortex to mix, and briefly centrifuge to pellet tissue. Carefully remove supernatant.**
   Remove supernatant by pipetting not to lose the tissue.

3. **Repeat the step 2 once or twice.**

4. **Add 180 ul of Buffer CL. Continue with step 2 of Tissue protocol on page 28.**

This protocol can be used with Clinic/Cell SV mini

J.

PROTOCOL FOR
DRIED BLOOD SPOT

Before experiment

- *Prepare absolute ethanol*
- *Prepare water baths or incubators to 56°C, 70°C and 85°C*
- *Prepare 1.5 ml microcentrifuge tube*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 56°C water bath*

* 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 any equivalent.)

1. **Place 3 ~ 4 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 200 ul of Buffer CL.**
   Use a 3 mm (1/8") single-hole paper puncher to cut out the circles from a dried blood spot.

2. **Incubate at 85°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
   Do not incubate for more than 15 min.

3. **Add 20 ul of Proteinase K, vortex to mix, and incubate at 56°C for 1 hour. Spin down briefly to remove any drops from inside of the lid.**

4. **Add 200 ul of Buffer BL 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 BL completely for efficient lysis. After addition of buffer BL, a white precipitate may be formed. This may be disappeared during incubation at 70°C and will not affect DNA recovery.

5. **Continue with step 5 of Tissue protocol on page 28.**

This protocol can be used with Clinic/Cell SV mini

K.

PROTOCOL FOR
GRAM NEGATIVE BACTERIA

Before experiment

- *Prepare water baths or incubators to 56°C and 70°C*
- *Prepare 1.5 ml microcentrifuge tube*
- *Prepare absolute ethanol*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 56°C water bath*

1. **Harvest cells (up to 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube by centrifugation for 1 min at full speed. Discard supernatant.**
   1 ~ 2 ml of overnight bacterial culture (A_{600}=1) may correspond to 1 ~ 2 x 10^9 cells.

2. **Resuspend completely the cell pellet in 200 ul of Buffer CL.**

3. **Pipet 20 ul of Proteinase K solution (20 mg/ml). Vortex vigorously to mix completely. Incubate 56°C for 15 min.**
   After complete lysis, lysis mixture will turn to clear from turbid. If the 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. **Spin down the tube briefly to remove any drops from inside of the lid.**

5. **Continue with step 3 of Tissue protocol on page 28.**

This protocol can be used with Clinic/Cell SV mini

L.

PROTOCOL FOR
GRAM POSITIVE BACTERIA

Before experiment

- *Prepare water baths or heating blocks to 37°C, 56°C and 70°C*
- *Prepare Lysozyme (LYS702, Bioshop, Canada, or equivalent) or Lysostaphin (L7386, SIGMA, USA, or equivalent)*
- *Prepare 1.5 ml microcentrifuge tube*
- *Prepare absolute ethanol*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 56°C water bath*

- **Prepare Enzyme Mixture** Resuspend the appropriate enzyme (not provided, listed below) with buffer GP just before use. Enzyme mixture should be stored at -20°C (or below) as small aliquots.; Ideally, once per an aliquot. Thawed aliquot should be discarded.

  30 mg/mL lysozyme (LYS702, Bioshop, Canada, or equivalent)
  or/and
  300 ug/mL lysostaphin (L7386, SIGMA, USA, or equivalent)

  * For certain species, such as *Staphylococcus*, treatment of lysostaphin (final conc.=300 ug/ml) may be required for efficient lysis instead of (or with) lysozyme. However, lysozyme is sufficient to lyse the cell wall for most Gram Positive bacterial strains

1. **Pellet cells (~ 2 x 10^9 cells) in a 1.5 ml microcentrifuge tube by centrifugation for 1 min at full speed. Discard the supernatant.**

2. **Resuspend the pellet thoroughly in 180 ul of the prepared enzyme mixture. Incubate at 37°C for 30 min.**
   The purpose of this treatment is to weaken the cell wall so that efficient cell lysis can take place.

This protocol can be used with Cell SV mini

3. ***(Optional :)*** **If RNA-free DNA is required, add 20 ul of RNase solution (20 mg/ml, Cat. No. 391-001) to the sample, mix well by vortexing and incubate for 2 min at room temperature.**
   Unless RNase A is treated, RNA will be copurified with DNA. RNA may inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 20 ul of Proteinase K solution (20 mg/ml, provided) and 200 ul of Buffer BL. Mix completely by vigorous vortexing or pipetting.**

5. **Incubate at 56°C for 30 min and then for a further 30 min at 70°C.**
   If any pathogen is subjected, it is strongly recommended that additional incubation at 70°C for 30 min should be substituted by at 95°C for 15 min.
   Longer incubation at 95°C will degrade DNA.
   After incubation, cool to room temperature.

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

7. **Continue with step 5 of Tissue protocol on page 28.**

M. PROTOCOL FOR YEAST

Before experiment

- *Prepare water baths or incubators to 37°C and 56°C*
- *Prepare the enzyme for lysing the cell wall. ; lyticase or zymolase*
- *Prepare 1.5 ml microcentrifuge tube*
- *Prepare absolute ethanol*
- *Equilibrate buffer AE to room temperature*
- *All centrifugation should be performed at room temperature*
- *Buffer CL and BL may precipitate at cool ambient temperature. If so, dissolve it in 37°C water bath*

1. **Add 3 ml (up to 5 x 10^7 cells) of a culture grown in YPD broth to a 15 ml centrofuge tube. Centrifuge at 5,000 xg for 10 min to pellet the cells. Remove the supernatant.**
   Alternatively, harvest twice in 1.5 ml or 2 ml micro-centrifuge tube. If 1.5 ml or 2 ml tube is employed, centrifuge at full speed (>13,000 xg) for 1 min, discard the supernatant and repeat again with the remain.
   When the value of A_{600} reaches to 1.0 (generally, log-phase), 3 ml of culture may yield approximately 10 ~ 25 ug of DNA .

2. **Resuspend the pellet thoroughly in 500 ul of Buffer YL.**

3. **Add 200 U of lyticase (Not provided) or 20 U of zymolase (Not provided) and gently pipet to mix completely.**
   Unit/mg of lyticase varies depending on the manufactures.

4. **Incubate at 37°C for 30 ~ 60 min to digest the cell wall.**
   Incubated cells turn to spheroplasts at this step, and this makes it easy the followed lysis step

5. **Centrifuge at 5,000 xg for 5 min. Remove the supernatant.**
   If 1.5 ml centrifugation tube is used, centrifuge at full speed for 1 min.

This protocol can be used with Cell SV mini

6. **Resuspend completely the cell pellet in 200 ul of Buffer CL.**

7. **Pipet 20 ul of Proteinase K solution (20 mg/ml). Vortex vigorously to mix completely. Incubate 56˚C for 15 min.**
   After complete lysis, lysis mixture will turn to clear from turbid. If the 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.

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

9. **Continue with step 3 of Tissue protocol on page 28.**

Troubleshooting for Exgene™ Blood/Cell/Clinic SV mini

Facts	Possible Causes	Suggestions
Low or no recovery	**Low cells in the starting sample**	Some samples may have low concentration of cells, and some whole blood may contain low concentration of white blood cells. Increase the sample volumes and load the SV column several times. Reduce the elution volume to minimum. When the cell mass is low, it is also recommended to use carrier NA (e.g. Poly-dN, glycogen, or tRNA dissolved at 20 ~ 40 ug/ml in buffer BL).
	Too many cells in the starting sample	Sample amount over the maximum capacity will lead to poor lysis, resulting in significantly low recovery. Reduce the amount of starting sample or increase the volume of buffers proportionally.
	Inefficient or insufficient lysis	Inefficient lysis may be due to several causes; - Insufficient mixing with buffer BL - Too much cells in the starting sample - Degenerated Proteinase K - Poor disruption of tissue After addition of buffer BL in protocol, vortex the mixture vigorously and immediately to mix completely. If too much cells present in the sample, reduce the starting sample volume, or increase the volume of buffer BL to double. Using tissue as sample material, lysis should be continued until the tissue is completely lysed. Completely lysed sample will not have any particulate in lysate. Proteinase K should be stored under 4°C for maintenance of proper activity. However, it is recommended to store in small aliquots at -20°C for prolonged preservation of its activity.
	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.

Troubleshooting for Exgene™ Blood/Cell/Clinic SV mini

Facts	Possible Causes	Suggestions
SV column has colored residue associated with it after wash, resulting in colored residue	**Insufficient lysis**	Insufficient lysis may cause that colored residue remains on SV column membrane. Repeat the procedure after consideration of 'Inefficient lysis' at "Low or no recovery"
	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 BW.
Column clogging	**Inefficient lysis**	Inefficient lysis may lead to column clogging. About inefficient lysis, check 'Inefficient lysis' at "Low or no recovery"
High $A_{260/280}$ ratio	**RNA contamination**	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, carry out RNase A treatment on protocol optionally.
Low $A_{260/280}$ ratio	**Insufficient lysis**	Insufficient lysis cause low DNA purity, and is due to insufficient mixing with buffer BL, too much cells in the starting sample, or degenerated Proteinase K. Check these out on next preparations.
	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 BW before washing with buffer TW.
Low concentration of DNA in eluate	**Low cells in starting sample (too high elution volume)**	Increase the volume of starting sample with additional volume of buffer used, and/or reduce the elution volume to 50 ul or do re-elution with eluate. For higher concentration of DNA in eluate, refer to the 'Elution' section of General considerations at page 12.

Facts	Possible Causes	Suggestions
Degraded DNA	**Starting sample is too old or mis-stored**	Too old or mis-stored sample often yield degraded DNA. Use fresh sample.
DNA floats out of well while loading of agarose gel	**Residual ethanol from Buffer TW remains in eluate**	Ensure that the TW wash step in protocol has been performed correctly. SV column membrane should be completely dried via additional centrifugation or air-drying. Refer the annotation of TW washing step.
Enzymatic reaction is not performed well with purified DNA	**Low purity of DNA**	Check "Low $A_{260/280}$ ratio"
	RNA contamination	RNA may inhibit some downstream enzymatic reactions. If RNA-free DNA is required, perform RNase treatment step optionally.
	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 BW.
	High salt concentration in eluate	Ensure that all washing steps were performed just in accordance with the protocols. Alternatively, carry out additional washing step with buffer TW. It may help remove high salt in eluate.
Precipitate in Buffer BL or CL	**Buffer stored in cool ambient condition**	For proper DNA purification, any precipitate in buffer BL/CL should be dissolved by incubating the buffer at 37°C or above until it disappears.

Ordering Information

Products	Type	Size	Cat. No.
GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*			
Plasmid Rapidprep	mini / spin	50	100-150
		200	100-102
GeneAll® *Exprep™* *for preparation of plasmid DNA*			
Plasmid SV mini	spin / vacuum	50	101-150
		200	101-102
		1,000	101-111
Plasmid SV Midi**	spin / vacuum	26	101-226
		50	101-250
		100	101-201
GeneAll® *Exfection™* *for preparation of highly pure plasmid DNA*			
Plasmid LE mini *(Low Endotoxin)*	spin / vacuum	50	111-150
		200	111-102
Plasmid LE Midi* *(Low Endotoxin)*	spin / vacuum	26	111-226
		100	111-201
Plasmid EF Midi* *(Endotoxin Free)*	spin	20	121-220
		100	121-201
GeneAll® *Expin™* *for purification of fragment DNA*			
Gel SV	mini / spin / vacuum	50	102-150
		200	102-102
PCR SV	mini / spin / vacuum	50	103-150
		200	103-102
CleanUp SV	mini / spin / vacuum	50	113-150
		200	113-102
Combo GP	mini / spin / vacuum	50	112-150
		200	112-102
GeneAll® *Exgene™* *for isolation of total DNA*			
Tissue SV mini*	spin / vacuum	100	104-101
		250	104-152
Tissue SV Midi**	spin / vacuum	26	104-226
		100	104-201
Tissue SV MAXI**	spin / vacuum	10	104-310
		26	104-326
Tissue *plus!* SV mini*	spin / vacuum	100	109-101
		250	109-152
Tissue *plus!* SV Midi**	spin / vacuum	26	109-226
		100	109-201
Tissue *plus!* SV MAXI**	spin / vacuum	10	109-310
		26	109-326

Products	Type	Size	Cat. No.
GeneAll® *Exgene™* *for isolation of total DNA*			
Blood SV mini	spin / vacuum	100	105-101
		250	105-152
Blood SV Midi**	spin / vacuum	26	105-226
		100	105-201
Blood SV MAXI**	spin / vacuum	10	105-310
		26	105-326
Cell SV mini	spin / vacuum	100	106-101
		250	106-152
Cell SV MAXI**	spin / vacuum	10	106-310
		26	106-326
Clinic SV mini	spin / vacuum	100	108-101
		250	108-152
Clinic SV Midi	spin / vacuum	26	108-226
		100	108-201
Clinic SV MAXI**	spin / vacuum	10	108-310
		26	108-326
Genomic DNA micro	spin	50	118-050
Plant SV mini	spin / vacuum	100	117-101
		250	117-152
Plant SV Midi**	spin / vacuum	26	117-226
		100	117-201
Plant SV MAXI**	spin / vacuum	10	117-310
		26	117-326
GMO SV mini	spin / vacuum	50	107-150
		200	107-102
GeneAll® *GenEx™* *for isolation of total DNA*			
GenEx™ B	Sx† / solution	100	220-101
	Sx† / solution	500	220-105
	Lx†† / solution	100	220-301
GenEx™ C	Sx† / solution	100	221-101
	Sx† / solution	500	221-105
	Lx†† / solution	100	221-301
GenEx™ T	Sx† / solution	100	222-101
	Sx† / solution	500	222-105
	Lx†† / solution	100	222-301
GeneAll® *DirEx™* *Single tube DNA extraction buffer for PCR*			
DirEx™	solution	50	250-050

Products	Type	Size	Cat. No.
GeneAll® *RNA Series* *for preparation of RNA*			
RiboEx™	solution	100	301-001
		200	301-002
Hybrid-R™	spin	100	305-101
Hybrid-R™ Blood RNA	spin	50	315-150
Hybrid-R™ miRNA	spin	50	325-150
RiboEx™ LS	solution	100	302-001
		200	302-002
Riboclear™	spin	50	303-150
Ribospin™	spin	50	304-150
Ribospin™ vRD	spin	50	302-150
Ribospin™ Plant	spin	50	307-150
Allspin™	spin	50	306-150
GeneAll® *AmpONE™* *for PCR amplification*			
Taq DNA polymerase	(2.5 U/μℓ)	250 U	501-025
		500 U	501-050
		1,000 U	501-100
α-Taq DNA polymerase	(2.5 U/μℓ)	250 U	502-025
		500 U	502-050
		1,000 U	502-100
Pfu DNA polymerase	(2.5 U/μℓ)	250 U	503-025
		500 U	503-050
		1,000 U	503-100
Hotstart Taq DNA polymerase	(2.5 U/μℓ)	250 U	531-025
		500 U	531-050
		1,000 U	531-100
Clean Taq DNA polymerase	(2.5 U/μℓ)	250 U	551-025
		500 U	551-050
		1,000 U	551-100
Clean α-Taq DNA polymerase	(2.5 U/μℓ)	250 U	552-025
		500 U	552-050
		1,000 U	552-100
Taq Master mix	0.5 ml x 2 tubes	2x	511-010
	0.5 ml x 10 tubes	2x	511-050
α-Taq Master mix	0.5 ml x 2 tubes	2x	512-010
	0.5 ml x 10 tubes	2x	512-050

Products	Type	Size	Cat. No.
GeneAll® *AmpONE™* *for PCR amplification*			
Taq Premix	96 tubes	20 μℓ	521-200
		50 μℓ	521-500
α-Taq Premix	96 tubes	20 μℓ	522-200
		50 μℓ	522-500
Taq Premix (w/o dye)	96 tubes	20 μℓ	524-200
α-Taq Premix (w/o dye)	96 tubes	20 μℓ	525-200
dNTP mix	2.5 mM each	500 μℓ	509-020
dNTP set (set of dATP, dCTP, dGTP and dTTP)	100 mM	1 ml x 4 tubes	509-040

* Each dNTP is available

** GeneAll® Tissue SV mini, Midi, and MAXI plus! kit provide the additional methods for the purification from animal whole blood.*
*** GeneAll® SV Midi / MAXI kits require the centrifuge which has a swinging-bucket rotor and ability of 4,000 ~ 5,000 xg.*
† On the basis of DNA purification from 300 ul whole blood, 2 x 10^6 cells or 10 mg animal tissue.
†† On the basis of DNA purification from 10 ml whole blood. 1 x 10^8 cells or 100 mg animal tissue.

Note

GeneAll® Exgene™ Brief Protocol

Brief mini protocol for tissue

** Before use this protocol, we strongly recommend you first read carefully the detailed protocol at page 27.*

Prepare..
56°C and 70°C water baths or incubators, absolute ethanol, 1.5 ml microcentrifuge tubes

1. Homogenize the tissue sample by an appropriate disruption method and add 200 ul of buffer CL.
(Otherwise, homogenize the tissue sample in 200 ul of buffer CL)

2. Add 20 ul of Proteinase K and mix completely.

3. Incubate at 56°C until completely lysed.

4. *(Optional)* Add 20 ul of RNase A to the sample for RNA-free DNA.

5. Add 200 ul of buffer BL to the sample and mix completely.

6. Incubate at 70°C for 10 min.

7. Add 200 ul of absolute ethanol and mix completely.

8. Transfer the mixture to a SV column and centrifuge for 1 min and discard the filtrate.

9. Add 600 ul of buffer BW, centrifuge for 1 min and discard the filtrate.

10. Add 700 ul of buffer TW, centrifuge for 1 min and discard the filtrate.

11. Centrifuge for 1 min at full speed and transfer the SV column into a fresh 1.5 ml tube.

12. Add 200 ul of buffer AE or sterilized water, incubate for 1 min and centrifuge for 1 min at full speed.

GeneAll® Exgene™ Brief Protocol

Brief mini protocol for whole blood and blood derivatives

**** Before use this protocol, we strongly recommend you first read carefully the detailed protocol at page 15.***

Prepare..
56°C water bath or incubator, absolute ethanol, 1.5 ml microcentrifuge tubes

1. Pipet 20 ul of Proteinase K into the 1.5 ml tube.

2. Transfer 200 ul of the sample to the tube.

3. *(Optional)* Add 20 ul of RNase A to the sample for RNA-free DNA.

4. Add 200 ul of buffer BL and vortex to mix completely.

5. Incubate at 56°C for 10 min.

6. Add 200 ul of absolute ethanol and mix completely.

7. Transfer the mixture to a SV column and centrifuge for 1 min and discard the filtrate.

8. Add 600 ul of buffer BW, centrifuge for 1 min and discard the filtrate.

9. Add 700 ul of buffer TW, centrifuge for 1 min and discard the filtrate.

10. Centrifuge for 1 min at full speed and transfer the SV column into a fresh 1.5 ml tube.

11. Add 200 ul of buffer AE or sterilized water, incubate for 1 min and centrifuge for 1 min at full speed.

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